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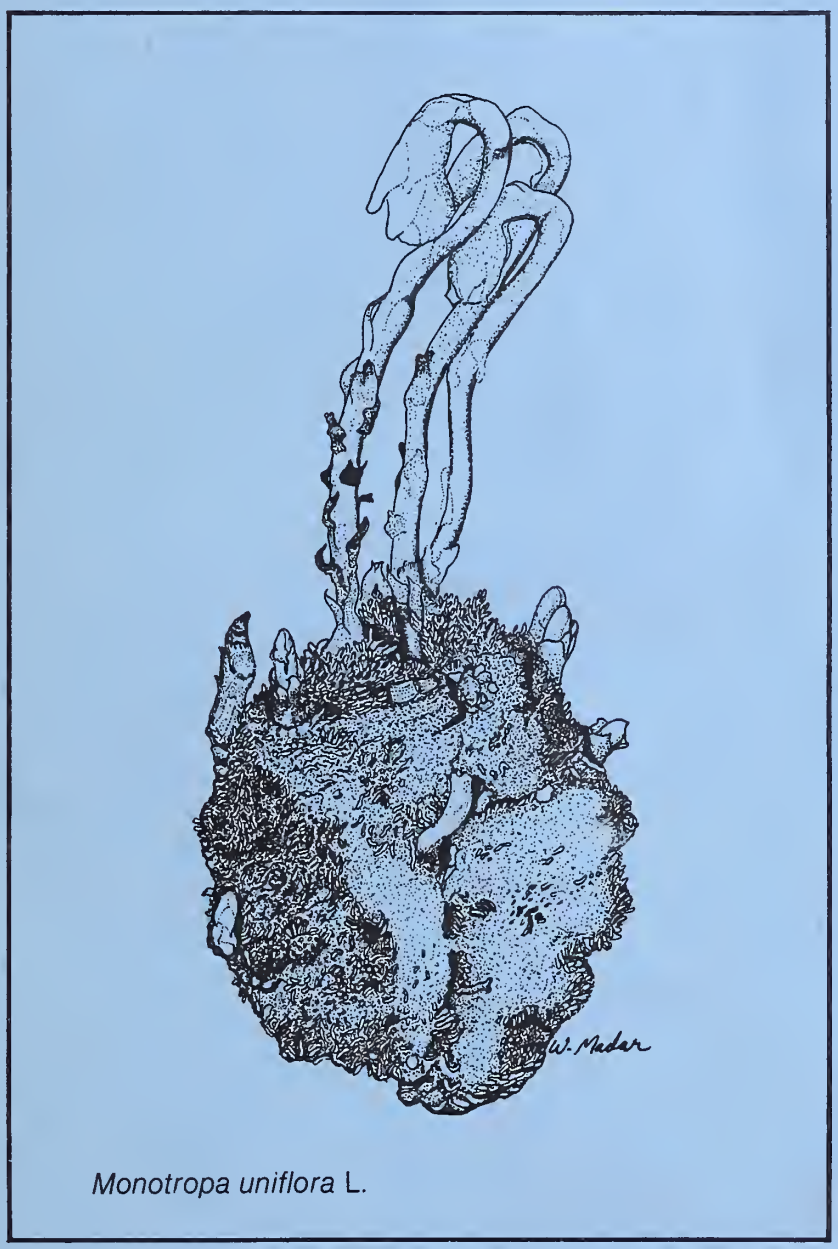
Proceedings of the

6th NORTH AMERICAN CONFERENCE ON MYCORRHIZAE



Monotropa uniflora L.

JUNE 25-29, 1984
BEND, OREGON, U.S.A.

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COMPILED AND EDITED BY RANDY MOLINA

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PREFACE

The 6th North American Conference on Mycorrhizae celebrated the approximate centennial of the discovery and naming of the phenomenon. The opening session was devoted to historical perspectives, as detailed in the first group of papers in the proceedings. The benchmark contributions by **F. Kamienski** (1882) and **A. B. Frank** (1885) are also included in English translation in the proceedings in recognition of their special place in the history of mycorrhiza science.

In further keeping with the centennial observance, the 6th NACOM was also dedicated to scientists who pioneered mycorrhiza research in North America:

G. F. Atkinson, Cornell University, described vesicular-arbuscular mycorrhizae of the Ophioglossaceae in 1894.

E. C. Jeffrey, University of Toronto, detailed the vesicular-arbuscular mycorrhizal colonization of Botrychium gametophytes in 1897.

D. T. MacDougall, New York Botanical Garden, published the first of a series of papers on physiology of mycorrhizae in 1898.

W. B. McDougall, University of Illinois, reported the first of his many studies on kinds and distribution of mycorrhizae in 1899.

The ensuing decades witnessed important contributions by many North American Scientists. One of these is **Dr. Kenneth D. Doak**, the dean of North American mycorrhiza researchers, who attended the 1st NACOM in 1969 and was with us at the 6th NACOM. Dr. Doak's publications on mycorrhizae, beginning in 1927, span nearly 3 decades. A second important contributor and collaborator with Dr. Doak was **Dr. A. B. Hatch.** Dr. Hatch, who also attended the 1st NACOM, had planned to attend the 6th but to our great sadness died in 1983.

More than 350 scientists, teachers, and students participated in the 6th NACOM, representing five Canadian provinces, two Mexican states, 40 U. S. states, and 22 countries outside North America. The conference was designed to encourage interactions between individuals in an attractive setting of Oregon's forests and mountains, and we feel that we succeeded in that. Part of the success is also attributable to sponsors that generously contributed towards covering the costs of invited speakers and providing amenities for all conference participants:

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Many faculty and students at Oregon State University and many employees of the U.S. Department of Agriculture, Agricultural Research Service and Forest Service, worked hard in organizing and carrying out the 6th NACOM. We leave them unnamed, but we are deeply grateful to them all!

Pam Henderson **David A. Perry** **Randy Molina**
Robert G. Linderman **James M. Trappe**

Organizing Committee, 6th NACOM

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THE PREHISTORY OF MYCORRHIZAE: A. B. FRANK'S PREDECESSORS

by

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Keywords: ectomycorrhizae, endomycorrhizae, VA mycorrhizae, orchids, Monotropa, bryophytes, mutualism

One of the several definitions of the word "history" found in most dictionaries is "the recorded events of the past." Prehistory, then, means those events that occurred before any record. For our purposes, we regard the history of mycorrhizae as starting with their description, interpretation, and naming by A. B. Frank (1885) (Fig. 1). The key element is the naming, for others had described and interpreted the phenomenon earlier. None of those interpretations, however, approached Frank's for its evidence, reasoning, and prescience.

Frank's first and ensuing papers gave rise to lively debate among botanists about who should be credited with the discovery of mycorrhizae. The two major contenders were Frank and F. Kamienski (Fig. 1), whose work in Poland had been published earlier than Frank's but was quite narrower in its scope and interpretation (Kamienski 1881, 1882). Even while asserting his claim to priority of discovery, Kamienski (1886) accepted Frank's terminology. Still earlier workers could be credited with what we now know to be good morphological descriptions of various types of mycorrhizae, and a few even braved conventional wisdom to hypothesize that this fungal colonization was beneficial for the host rather than pathogenic.

Our interest is not to attempt the impossible and irrelevant, i.e., deciding priority of discovery and correct interpretation of the mycorrhizal phenomenon. Rather, we present the information and ideas that unfolded in the scientific literature before Frank's papers appeared. In so doing, we hope to reveal a bit of the flavor of the times and to recall that we later researchers owe much to our predecessors. The story also instructs us that new truths are revealed through hard evidence and sound but innovative thinking, not through the conventions of the times. The material has been reviewed and discussed in detail before (Rayner 1926, Hatch 1937, Kelley 1950). We choose, in contrast, to let the early authors speak for themselves in their original English or in translation from the original Latin, French, Italian, or German. Our commentary mostly provides transitions or summarizes or condenses material that is too lengthy to quote in full. Because of the particular importance of the papers by Kamienski (1882) and Frank (1885), the former is presented in partial translation and the latter in full translation after our paper. A good many of the early authors did not contribute new ideas or interpretations; we include only those who expanded dimensions of observation or thought.

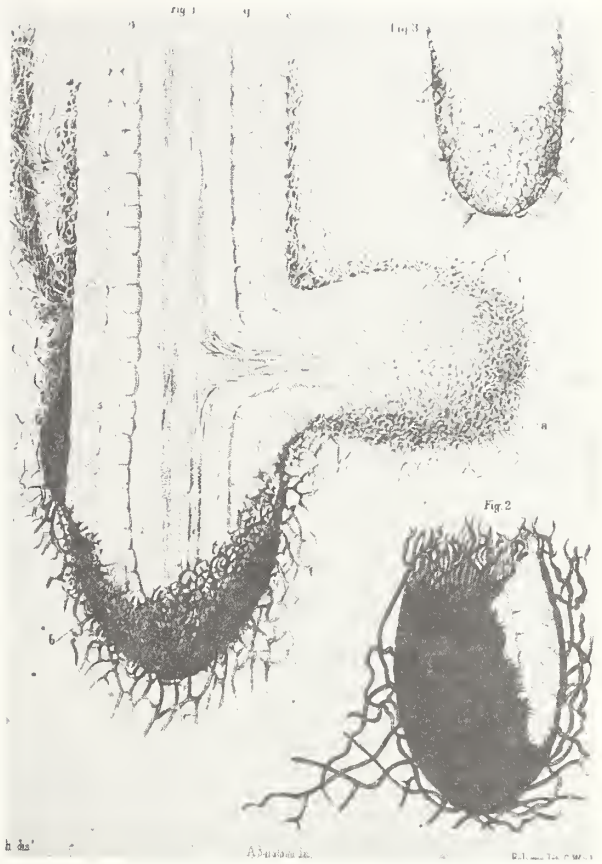
Translation, even of scientific works, is as much art as science. We often had to choose between a literal rendering of 19th century expression on the one hand and clarity for the 20th century on the other. One can provide so much "flavor of the times" that the story hardly makes sense. Where the choice had to be made, we chose clarity. Thus, for example, Vittadini's (1842) "spongiola" and Gibelli's (1883) "spugnola" are given in English as "feeder root" rather than the literal "little sponge," Schacht's (1854) "Pilzfäden" as "hyphae," not as "fungus threads." Sentences of paragraph length with mazes of subordinate phrases and clauses, especially popular with certain German authors of the time, are often broken into shorter, more digestable sentences in our English version.

As is common in human affairs, the sequence of discoveries and reports was somewhat disorderly: a description of an ectomycorrhiza here, colonization of orchid rhizomes or liverwort thalli there. We keep that disorder by presenting the material in chronological order, regardless of the specific content of the papers. This, perhaps more than anything else, reveals how many workers observed mycorrhizae independently before one of them succeeded in putting the data together in a comprehensive and interpretable way. To aid the specialist interested in a special history, however, we summarize the sequence by mycorrhiza type at the end of our paper.

THE 1840'S

Structures of probable mycorrhizal origin were sketchily described from roots and variously misinterpreted prior to 1840, perhaps first by Meyen (1829). Elias Fries, the great Swedish mycologist, has been said to be "the first to recognize the fungal nature of the outer covering of Monotropa roots" (Hatch 1937). The paper cited (Fries 1832) deals, however, with a smut that parasitized Monotropa stems and roots, Urocystis (Tuburcinea) monotropae (Fries) Fischer. The first illustration of a mycorrhiza, as it happens an ectomycorrhiza, appeared in print in 1840 in Theodor Hartig's "Complete natural history of the cultivated forest plants of Germany." His credentials were listed as "Ducal Braunschweig Forest Councilor and Professor, member of the Imperial Leopold Academy of Naturalists, the Society of the Friends of Natural Philosophy of Berlin, the Royal Swedish Physiographic Society of Lund, and the Societies for Natural Philosophy and Technology of Berlin, the Harz, Konigsberg, Marburg, Potsdam and Stettin."

Hartig illustrated roots of various types, describing them in detail in the captions, for example in Plate 18, Figure 4: "Fine roots of pine in spring, natural size. a. Primary roots, i.e., that part of the fine roots responsible of lengthening and broadening of the root system. b. Absorbing roots, i.e., the much branched,



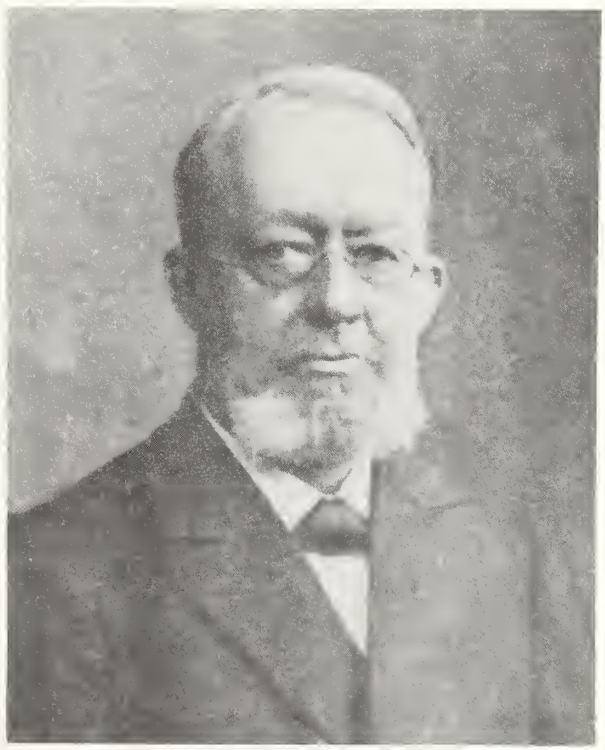


Figure.1 Upper half reproduced from drawings of G. Gibelli (1883).
Lower right F. M. Kamienski. Lower left A. B. Frank.

finest root branches that sprout from the primary roots. Over winter, the tip of each of these is cloaked with brown filaments; in spring, a thick, asparagus-like shoot, the true organ of below-ground plant life, develops. This 'asparagus tip' develops to mid summer and seems then to shrink, in that the outer, very thick cell mass dies, dries, and becomes brown, whereupon the youngest part of the absorbing root is no longer distinguishable from the primary root tip."

In Plate 18, Figure 9, Hartig (1840) illustrates a longitudinal section of a pine ectomycorrhiza, but the caption indicates that he did not recognize the fungal component for what it was, referring to his clearly drawn fungal mantle as a "persistent periderm" and the intercellular fungal network as "a peculiar wall structure." His analysis in total reads: "The outermost covering forms a persistent periderm from radial cells; the periderm encloses the large-celled parenchyma by means of a peculiar wall structure. It shows here, namely, the walls of the outer cells as as a leaf-vein-like netted, anastomosed plexus. In Figure 10 I represent the ramified intercellular veins that encompass the entire circumference of the cells with the possible purpose of enhancing cell activity. This organization, to my knowledge not observed heretofore, directly attains in this sense a special physiological significance. This lymphatic cell weft lives only in summer, then dries and becomes a thin brown cell layer covered with long-stretching, filament cells with two nuclei that contain spiral element bundles."

Theodor Hartig's illustrations are diagrammatic and not particularly elegant, his descriptions brief and incomplete, and his interpretation wrong. Nonetheless, he was the first to observe and report the ectomycorrhizal mantle and intercellular "Hartig net," an accomplishment overlooked by other workers until his son, Robert Hartig, also a respected botanist, resurrected it in 1886.

F. Unger (1840) described the intimate relationship between roots of Monotropa hypopitys and Picea abies (p. 29): "The individual, rhizome-like, tuberous, irregularly shaped body from which the flowering shoots arise consists of a bundle of intimately interwoven rootlets which belong in part to the parasite and in part to the host plant... This rootlet mass... becomes so compact near the center that soil particles are virtually excluded to leave only a mass of rootlets that cannot in any way be disentangled. The contact between host and parasite roots, which are easily distinguished in color, form, and consistency, is intimate... however, haustoria or similar organs that would establish a direct union, a penetration of one by the other, are absent. This circumstance can, of course, lead to the presumption held earlier that no parasitism is involved here, because the feeder roots of the tree do not die, and the Monotropa rootlets interwoven with those of the tree extend into the soil. We perceive, by virtue of the intimate root contact of the two plants, an exudation on the part of one and an ensuing absorption of the nutritious exudate on the part of the other, and therefore conclude that a genuine dependent relationship of both must certainly take place here." In an accompanying plate Unger clearly shows a differentiated outer mantle on the Monotropa and Picea rootlets, although the magnification is insufficient to make out the anatomy. His suggestion that Monotropa obtains nutrients indirectly from tree roots, though not based on the fungal connection between the two species, is closer to being correct than are other hypotheses of the times.

In England, meanwhile, an exchange of observations and opinions on the nature of Monotropa roots was begun in the fledgling journal, The Phytologist (because of a misprint on the title page of "Volume the First," the papers involved are sometimes cited as appearing in 1844 rather than their actual time of publication, 1841 and 1842). These writings have been reviewed adequately by Rayner (1926) and Hatch (1937), so we will note only the two that proved to be particularly relevant. Edwin Lees (1841) described the root system of Monotropa hypopitys and associated beech roots much the same as had Unger (1840) but added a critical new observation (p. 100): "...the whole (root) mass is obscured with a hirsuture that appears like a byssoid fungus. These hairy fibres, however, appear to me to be really part of the economy of the plant, imbibing nutriment from the rootlets of the beech, to which they are closely applied, and conveying it to the succulent radicles of the Monotropa, with which they are also connected. In proof of this I find these hairy fibres closely applied to the swollen rootlets in the smallest plants that I have been able to find... a close examination shows ultimate hairy fibres fixing the roots of the Monotropa to the rootlets of the beech and seeming to absorb nutriment from the alburnum of the latter." Lees thus seemed to back away from regarding the "hairy fibres" as fungal, but he came close to being the first to propose that a mycorrhizal fungus provides nourishment to its host and that achlorophyllous plants are epiparasites on associated trees by way of a shared mycorrhizal fungus, a hypothesis that was first experimentally verified nearly 120 years later by Björkman (1960). Thomas Rylands (1842) followed up Lees' work to demonstrate convincingly that Monotropa roots are indeed invested with mycelium. Unfortunately, his observations and the wisdom of the times caused him to conclude (p. 343): "Such are the data on which I would ground the opinion that the 'byssoid substance' is really fungoid, and performs no essential function in the economy of the Monotropa."

It befell the mycologists to recognize the fungal involvement in ectomycorrhizae, as prominently seen in the proliferation of rootlets around fruiting bodies of several Elaphomyces spp. (hypogeous Ascomycotina). The brothers Louis-René and Charles Tulasne (Tulasne & Tulasne 1841) detailed the intimate relationship between tree rootlets and Elaphomyces granulatus, although they did not mention a fungal mantle on the rootlets

themselves (p. 7): "Most often mature specimens of this species are completely enveloped in a tight but not thick covering, elegantly woven by the fine rootlets of neighboring trees; these rootlets are slightly enlarged by being flattened onto the surface of the fungal sporocarp, in contrast to rootlets of the same source growing out into the soil. Evidently, they develop in a particular way characteristic for the fungus that they embrace so intimately. These rootlets seem to branch from a single root, often so close to the fungus that the fungus itself seems to originate from the root. Moreover, the root that produces these rootlets is distant enough from its parasitized parent plant that the connection is difficult to trace. We say parasitized, because we would not know how to defend the proposition that our fungus does not take something from the roots that surround it. Though this opinion perhaps has not been positively confirmed, the numerous facts that we have gathered lead us to term it parasitism, besides which this is the ordinary condition of the lower plants in general, such as those we are concerned with here. We point out that the sphere in which E. granulatus is enclosed is always composed of a single species, and that these roots are alive while the fungus lives but weaken and die when the fungus dies."

The next year, Carlo Vittadini (1842) presented quite a different conclusion about the rootlets associated with Elaphomyces sporocarps (p. 76): "The sporocarp is covered with a thick, earthy-fibrillose, pale tawny crust. This crust separates easily from the peridium, and its inner part is seen to contain rambling fibrils or rootlets of various plants. These rootlets bear innumerable root tips, which are colonized (by hyphae originating) from among the warts of the peridium, and which without doubt remove fungal products... The plant rootlets, which by no means rarely cloak the surfaces of Elaphomyces and pervade their thalli in various ways, remove materials rather than nourish the fungus, contrary to the opinion of the Tulasnes. The rootlets are certainly absent from many Elaphomyces species, are always absent in the initial developmental stage of the fungus, develop on the developing sporocarp, and perish along with the death of the sporocarp. Add to this that the fungi, especially the higher groups, seldom parasitize living, growing plants. It seems to me totally inconsonant with all these evidences to suppose that plants parasitized by a fungus would extend their rootlets around that fungus so as to be better exploited by it. Furthermore, we know that plants extend and multiply their rootlets where they find abundant nutrients. Willow roots submerged in water exhibit this strikingly. It is our decided opinion, that beyond all doubt the higher plants absorb nutrients from the fungus by their feeder rootlets." Vittadini is thus the first to unequivocally state that the roots derive nourishment from the fungus. His view, of course, was not one of mutualism, in that he excluded the possibility of the fungus also deriving nourishment from the roots. Researchers in mycorrhizae and other branches of mycology are indebted to Vittadini for another reason: he was the first to solidify pure culture media with gelatin (Ainsworth 1976).

The early 1840's also witnessed the first account of apparent vesicular-arbuscular mycorrhizae by Karl Nägeli (1842, p. 278): "While examining roots of several Iris spp. last autumn, I rather frequently observed fungal structures within old cells. It is known that Iris roots consist of a central vascular bundle and a large-meshed parenchymatous cortex, as are roots of all other monocots. It was in these parenchymatous cells that three species of fungus occurred. One of the fungi... is found particularly in cells next to the vascular bundle; it usually fills them, as does a granular brown mass at the same time. One finds it also... all the way to the epidermal cells... The second fungal species has a clustered form, because its branches are short and closely packed. Its walls, initially gelatinous, eventually become a handsome yellowish brown." The structures thus described by Nägeli seem easily equated with arbuscules, some in stages of digestion by the host cell. His third fungus is described in a way that makes it hard to know what he was seeing, but its final developmental stage could be an intracellular vesicle. Nägeli detected no entry of these fungi into cells and could not determine their origin. The evidence at hand, however, was inadequate in his view to demonstrate origin by spontanous generation.

Five years passed before the next addition to mycorrhizal phenomena appeared in print. Siegfried Reissek (1847) presented the first description of orchid mycorrhizae in which the endophyte was recognized to be a fungus. He detailed the developmental morphology of mycorrhizae of Neottia nidus-avis, although he seemed to have the sequence backward, i.e. what is now understood to be the amorphous material resulting from hyphal digestion he regarded as the source of the hyphae. He also investigated species of Orchis, Gymnadenia, and Ophrys of the European natives as well as tropical species in several genera (p. 37): "In all species that I studied, at least an intimation of this structure existed, thus for example in the elongated aerial roots of Vanilla plantifolia.... In general the fungus structure is abundant in belowground roots, less abundant at the surface, and very infrequent in aerial roots, which are exposed to light." As generally the case with his contemporaries, Reissek was unable to break from conventional thinking in his interpretation of the phenomenon (p. 38): "The regularity and constancy with which the fungal structures occur in the orchids must for those reasons be viewed as a legitimate and vital phenomenon. It is to be conceded, that the presence of the fungus should be quite unessential for the existence and growth of the plant; their constancy and universality stamps them nonetheless as the rule. It is not conditional for the life of the species, but still for the species it is a conditional rule. It is a rule which one can in certain ways compare to the flower production of phanerogams. Just as the majority of phanerogams can continue existence and grow without production of flowers, so also can the orchids produce without the root fungus."

Reissek was perhaps the first to try to grow orchid endophytes (p. 40): "To induce spore production by the fungus... some fine roots of Orchis morio, not detached from the plant, were cut longitudinally, and some were torn apart with the tip of a knife or needle. The stem, rhizomes, and roots of the plant were wrapped in damp fly paper and placed in a half-dark place. Many fungi exposed by the cutting and tearing of the roots and brought into contact with the air could freely develop in the surrounding moist atmosphere. After two to four days (in autumn), a delicate, mold-like flush of the root fungi began to grow out from the root." He then describes the fungi, but their identity cannot be determined from the descriptions, nor can it be sure that mycorrhizal fungi were observed rather than contaminants. Still, Reissek used a method that was ancestral to the maceration methods now used to isolate orchid endophytes.

THE 1850'S AND 1860'S

After considerable activity--discovery, description, speculation--on root endophytes in the 1840's, the next two decades were rather quiet. Neither Nägeli (1842) nor Reissek (1847) had been able to determine how endomycorrhizal endophytes got into host cells and toyed with (but did not espouse) thoughts of spontaneous generation. Hermann Schacht (1854) repeated some of their observations and put to rest any consideration of spontaneous generation (p. 618): "The presence of articulated filaments within apparently uninjured plant cells has been variously interpreted. Such filaments have become held as either independent plants, indeed as fungi, and thus evidently as support for the hypothesis of spontaneously generation (Nägeli, Reissek), or they have become considered as dependent structures--abnormal, morbid products of the cell in which they occur (Schleiden and others); finally, some have now and then regarded them as a special vascular system within the determined cells (Göttsche, Schleiden). In my researches, the above mentioned filaments have frequently appeared within apparently intact cells; I have recognized the fungal nature of these in all cases with utmost certainty and at the same time have demonstrated positively that the filaments penetrate the cell from the outside and cross from one cell to another, often extending deeply within the plant part. The support that scholars appear to obtain for spontaneous generation through these fungi thereby is fallen."

Schacht (1854) also corrects erroneous conclusions of some earlier workers, that the endophytic structures in orchids and bryophytes were actually "a peculiar kind of vascular system" (p. 619): "In both Pellia and Preissia the fungus never appears in young thalli, but in autumn one seldom misses it in older thalli. In Preissia the hyphae, which wander from one cell to another, form large, globose, sometimes detaching swellings, which not infrequently fill the cell and in turn again grow out in narrow hyphae. In Pellia, however, they form a whole series of small, pearl-like swellings which in time again send out long hyphae. The cell wall in both Pellia and Preissia has small, thinned places or so called pore canals; the fungus seems to find its way through these. Large amounts of hyphae are found in the soil on which both liverworts grow. Similar hyphae occur not only in Neottidium but also in rhizomes and lateral roots of other orchids; I found them in rhizomes of Epipigium and Corallorhiza, moreover in lateral roots of Goodyera and Limodorum. As with the liverwort fungi, these seem never to occur in the younger parts of the rhizomes or the young lateral shoots." Schacht then proceeds to describe death of orchid cortical cells and coagulation of starch granules in the roots, compares these phenomena with rotted potato tubers, and concludes that in orchids the fungus is pathogenic.

Confusing mention of ectomycorrhizae, the first since that of Vittadini (1842), appears incidentally in a paper by Gasparinni (1856): "...we have discovered, on a chestnut tree (Castanea vesca Lin.), very young, shallow rootlets that divide and subdivide in brown, cylindric branchlets with a roughened epidermis nippled by obtuse root tips that sometimes appear clavate on account of the blackish hair-roots. Some of the tubular filaments around these seem to me to be produced by a mold or by other cryptogams." Gasparrini describes "filaments" around feeder roots of Arbutus, Corylus, and Pinus spp., but he seems not to have figured out the connection with the roots. He does note that root hairs are often lacking when abundant filaments are present on these hosts.

Returning to the orchids, Eduard Prillieux (1856) confirmed many of the observations of Schacht (1854) but differed in his interpretation of effects on the host (p. 273): "The examination of Neottia nidus avis has convinced me of the accuracy of Schacht's interpretation, but I think that the fungal mycelium penetrates living cells; I have seen and drawn it inside cells that contain large nuclei in a still young part of the root where the cells had not attained full development. Beyond that, the appearance of these filaments is certainly that of a fungus; there is a proof that seems to me entirely convincing. If one digs up an individual N. nidus avis from a sandy soil such as that of the Saint-Germain forest, one notices that the sand grains remain clustered around the root mass, whereas further away they are loose. It is difficult to remove this encompassing soil from the roots. When one resorts to the microscope to find the explanation of this phenomenon, one easily sees numerous filaments identical to those contained within the plant tissue. One cannot doubt that it is the mycelium of a fungus that runs through the soil and binds the sand grains. Consequently, it seems impossible to deny that this fungus penetrates the plant and multiplies within its cells."

THE 1870'S

Seventeen years then elapsed before new reports on mycorrhizae appeared. J. Reinke (1873) defined the method of passage from one cell to

another in Corallorhiza innata (p. 162): "...the cells lying near the upper surface are generally quite filled with fungus, which conglomerates into clusters within the cells. I have never observed reproductive structures on these hyphae. Their growth from one cell to another seems to be by boring through the cell wall... the mycelium mentioned above is found in these outermost cell layers as well as in the outer layers of the mucilagenous cell region without, so it seems, much harm to the vital activity of the rest of the organism, or else the plant would go to ruin when the mycelium completely spreads through the cortex." Oscar Drude (1873) found Neottia nidus-avis to have mycorrhizae similar to those described for Corallorhiza by Reinke and concluded that Neottia roots draw nutrients from organic matter of the forest floor. He regarded the fungus as strictly parasitic. He did not detect the fungus of Monotropa mycorrhizae and felt that Monotropa hypopitys directly parasitizes roots of Fagus, Pinus, and Abies.

The Hartig net of ectomycorrhizae had gone virtually unnoticed from its original description by T. Hartig (1840) and was thus still not realized as being of fungal origin. Hellmuth Bruchmann (1874), in a brief section on dichotomy of pine roots towards the end of a long paper on roots of Lycopodium and Isoetes, incidently mentions this startling, new perception (p. 572): "The cortex of all dichotomous roots was interlaced with a mycelium; I was unable to find mycelium in the central cylinder. The root hairs failed, as in Cycas, and instead the root tip as well as its whole exterior was bedecked with a crust composed of dead cortical cells and mycelial weft... The same mycelial tissue found in the cortical tissue as well as in the rudimentary root epidermis and the crust that surrounded the root body was likewise present on other, unforked roots found in the same soil."

H. G. Holle (1875) reopened the discussion of vesicular-arbuscular mycorrhizae in studies of ferns (p.247): "In the inner, starch-bearing layers of the outer cortex occurs fungal mycelium, as already mentioned several times in the literature. In Ophioglossum it appears in almost every root, although I could perceive no trace of it in some strong roots. Also in Botrychium lunaria it was present and luxuriously developed in specimens I examined; in B. rutaefolium it is found mostly only in the diarch roots or at least is more strongly developed in these. Since we can hardly assume that the fungus has a special preference for such roots, it seems closer to the truth to suppose that the fungi, having already penetrated the quite young root, depress its developmental capability in a way that its central cylinder forms not as triarch but as diarch, the more so in that the fungi also exert a degenerating influence on the root, as the cells attacked by them only slightly thicken their walls."

The ectomycorrhizae associated with Elaphomyces spp., discussed by the Tulasnes (1841) and Vittadini (1842), were revisited by Emile Boudier (1876) 35 years later (p. 115): "All mycologists who have collected Elaphomyces spp. with a yellow peridium, such as variegatus, granulatus, and its variety asperulus Tul., must have noticed that the small cavity in which the fungus occurs is lined with a reddish network that closely resembles mycelium but is formed by the fine roots of neighboring trees and shrubs (birch, oak and chestnut)... it can be attributed to a special effect of the mycelium on the roots. In fact, if one examines the rootlets with care, one sees that they are turgid, irregular, and branched in an abnormal manner, and their color is less red and more yellow than is usual. Moreover, they are so numerous and so pressed together and interwoven that they occupy all the spaces between the small warts on the surface of the peridium of the fungus. With a hand lens, one can observe that this network of rootlets is mantled by a thin layer of orange or yellow hyphae, especially if the specimen is not too wet. If one then examines a segment of rootlet, or better yet a thin section, under the microscope, one immediately perceives that the outer part is invaded by very fine, septate, yellow hyphae... The mycelium of these Elaphomyces occurs principally on the fine roots, probably as a parasite, because it modifies them, forcing them to develop abnormally and to appress themselves against the peridium, which must draw from them the nutrients it needs to develop. Even though the mycelium invades the fine roots, it penetrates and attacks only the outermost layer of cells, leaving the interior healthy. Moreover, it does not diminish root vitality; on the contrary, it activates them while inducing an abundance of nutrient sap... I have rather frequently encountered other hyphae on roots... that I believe to belong to Cenococcum geophilum, which is so common in these localities." So, the ectomycorrhizal mantle and Hartig net finally are revealed as fungal structures, first briefly by Bruchmann (1874) and then in more detail by Boudier.

Boudier's interpretation of the relationship between fungus and host is half right--nourishment of the fungus by the host; Vittadini (1842) had also been half right--nourishment of the host by the fungus. Neither postulated mutualism. W. Pfeffer (1877) was the first to publish this philosophical leap, in special reference to orchids but as an aside in a long paper on insectivorous plants (p. 997): "A fungus seems to occur commonly in the rhizome and root of saprophytic orchids. Its mycelium is found in living cells, at least in Neottia nidus-avis, and from these it sends hyphae to the outside. The hyphae contact organic and inorganic soil particles and indeed grow around them in a way similar to that of root hairs (Prillieux 1856, p. 272). In this situation one cannot help but suppose that an association is formed in which the host orchids also extract nourishment, in that they receive nutrients taken up from the soil by hyphae of parasitic or saprophytic fungi. According to Prillieux for Neottia, the hyphae could to a certain extent compensate for the constant lack of root hairs and, for other orchids, could function in conjunction with root hairs. In

Neottia the fungus seems to be a constant guest; at least, Prillieux invariably found it, and I also have always found the hyphae in all the Neottia plants that I have studied from diverse regions. According to Prillieux, Schacht, and others, as well as in my own experience, hyphae occur constantly in other saprophytic orchids. To be sure, I am convinced that these orchids can also exist without such fungi; meanwhile, it is not precluded that the hyphae, where present, bring nourishment to their host."

While Pfeffer (1877) proposed the idea of mutualism in fungus-root associations, Albert Bernard Frank (1877) presented much more convincingly a similar concept for the fungusalga association of crustose lichens. Frank proposed the term "Symbiotismus," i.e., a living together in which both organisms benefit from a balanced relationship. This term was later taken up (as "symbiosis") and elaborated upon by de Bary (1879), who is often thought to have been its originator.

1880 to 1885

Feeder roots proliferating around sporocarps of the truffle genus Elaphomyces continued to elicit interest in the 1880's as they had in the preceding four decades. Max Reess (1880) confirmed the ectomycorrhizal mantle and Hartig net as described by earlier workers and noted that fungi other than Elaphomyces spp. do the same kind of thing with pine roots. With no reference to Vittadini's (1842) assertion that the rootlets draw nourishment from the root, Reess simply questioned whether the fungus totally depends on parasitism of the root for its nutrition or whether it has some saprobic ability as well. After Frank's (1885) landmark paper, Reess (1885) claimed, in a way, some unpublished priority of discovery (p. 293): "...I referred... to Kamienski's work and emphasized that my researches on Elaphomyces revealed analogous root fungi, probably of quite diverse species, both on conifers and on other roots. I have several times debated the view now expressed by Frank on the significance of certain of these root fungi; I would not publish on this, however, because the general occurrence, indeed the very existence of the entire phenomenon had already been searchingly treated by Kamienski... the results of my anatomical and developmental studies on Elaphomyces and pine--with necessary modifications--agree with Frank's statements, including the points which I had not touched upon in my contribution in 1880 but since had worked through."

Franz Kamienski (1881) announced his work on fungal endophytes of Monotropa hypopitys in a preliminary report. He describes Monotropa morphology in much the same terms as preceding observers did and the anatomy in new and accurate detail. In reference to the roots and the meaning of his observations, he wrote (p. 460): "Monotropa produces no haustoria by which it could be characterized as a parasite; it is no parasite, but an achlorophyllous, humus-dwelling plant, namely a saprophyte. The parasitic connections with roots of Abies excelsa, which Drude described and illustrated, are nothing more than small, strongly dichotomously forked fir roots deformed by a parasitic fungus which is interwoven with the habitually very similar Monotropa roots. Without exception, all Monotropa roots examined were invested with a similar or most likely the same fungus. The mycelium of this fungus forms a closed, dense, thick layer that completely envelops the root tip and especially the active part of the root; this mycelial layer always follows along with the growth of the root mass, so that only a few destroyed epidermal cells are ever exposed. This fungus grows only on the surface of the epidermis and sends neither hyphae nor haustoria into the root tissue. On these grounds as well as from the fact that all growing specimens examined to date, from various depths and types of soil, are completely the same and healthy, I judge that Monotropa is not serving as a host plant for these fungi, but rather only as an apparent underlying support. If we now consider the nutrition of Monotropa as a saprophyte and ask in what form and in what manner the nutrients reach the root from the surrounding humus, we would see that in the specimens studied it can happen only through the above mentioned mycelial layer, because here not one root exists that directly contacts the humus and can extract nutrient directly from it. These fungi must also play the role of a mediator in nutrient uptake."

Kamienski's more detailed report (1882) is presented in full translation after our paper, so it need not be detailed here. He regarded the Monotropa function as primarily providing a physical base for the fungus, which in turn substitutes for root hairs in providing nutrients to the Monotropa. He hypothesized that the same fungi parasitize the nearby rootlets of associated trees and somewhat ambiguously implies epiparasitism of the Monotropa on the trees via the shared mycelium. He nowhere suggests mutualism between tree and fungus.

Ectomycorrhizae received the most detailed morphological and anatomical study yet by Giuseppi Gibelli (1883) in studies on the "ink" disease of chestnut. Gibelli's beautiful and accurate illustrations of ectomycorrhizae are unsurpassed to this day (Fig. 1); the details that he describes and illustrates leave no doubt that one of the several ectomycorrhizal types he studied was formed with Cenococcum geophilum Fr. He applied one term still used in ectomycorrhizal morphology (p. 301): "The thickest of the rootlets are grouped in tubercles formed by an infinity of short branchlets and many recurvings, disposed on all possible planes. These are very reminiscent, in small dimension, of the shape of red coral clusters, so this form can be designated by the term 'coralloid.'" Gibelli found ectomycorrhizae on species of Quercus, Corylus, Ostrya and Carpinus. He regarded the condition as parasitic but probably with little or no impact on tree growth; he did not consider it to be a cause of the chestnut "ink" disease, because it occurred commonly on other tree genera not thus afflicted.

An exemplary anatomical study of orchid mycorrhizae was produced by Albert Mollberg (1884), who first recorded and accurately interpreted many of the structures and phenomena involved (p. 526): "From the structure of the hyphae, we must suppose that various fungal species occur in our orchids. The mycelia in the rhizomes of Corallorhiza innata are very different from all the others. Through the characteristic formation of clamp connections, they reveal themselves as Basidiomycetes. Clamp connections fail in the rest of the species... The appearance of the infected cells is essentially unchanged by the parasite. Even growth of cells in the apical region of the root, where the fungus sometimes appears and where the cells have not yet reached their definitive size, is not in the least hindered by the parasite: growth in length and wall thickness are not hindered, also the specific thickenings of various kinds are in no way abnormal. The nucleus and the protoplasmic bodies experience no evident disorder." Mollberg described response of host cell nuclei to the fungus and the constriction of hyphae where they pass through cell walls. He observed the sheathing of the hyphae by a cellulose membrane; although he supposed it unlikely that the host laid this membrane over the fungus, he leaves the question open to further research.

Having detailed orchid mycorrhizae, Mollberg (1884) wondered about other monocots (p. 533): "The general distribution of fungal mycelium in roots of all of our orchids induced me to investigate if other plants growing in the same sites might not also show analogous phenomena. In my excursions, therefore, I collected such plants for study from the immediate neighborhood of orchids. Among the many plants studied, I found only a few which accommodated fungi in their roots, but their mycelia exhibited different form and spread. Thus I found in long roots of Arum maculatum L. mycelium of irregular development interspersed through the various layers of the fundamental tissue. The mycelium was little branched and did not fill the cell but grew directly from cell to cell. The individual hyphae are very broad, about twice as thick as the common orchid fungi, and possess clavate swellings at their tips as well as considerable intercalary broadening. Their primary differentiating character, however, lies in that they are distributed both inter- and intracellularly. The same properties held for the mycelium that I found in Colchicum autumnae L. and Allium scorodoprasum L."
In conclusion, Mollberg kept an open mind on the meaning of the endophyte in orchid roots, pending further research (p. 535): "The young plants developed from seed acquire the parasite very early, but they develop further without problem, so generally the well-being of the plant seems unimpaired through its presence; leaves and flowers develop normally and a look into the interior of the root structures shows no deformation. An injury of the host plant by the invader likewise is not perceptible; my observations do not permit conclusions, however, as to whether the hyphae bring nutrients to their host as Pfeffer (1877) supposes. Comparative culture experiments with young, fungus-free and fungus-affected plants can alone lead to a conclusion to the question hereby raised."

Lycopodiaceae received further attention in 1884 by M. Treub, who described the colonization rather briefly (p. 124): "Still, if one sees... primary tubercles of rather old prothallia still lacking root hairs,... it is because the peripheral cells of the tubercles all enclose hyphae of an endophytic fungus, probably belonging to the genus Pythium. The cells in which this Pythium are found seem incapable of developing root hairs. The endophyte later also spreads into the part of the prothallium above the primary tubercles. What is remarkable is that in the peripheral cells the fungal hyphae are found in the cell lumen, while near the interior of the prothallium they remain almost exclusively between the cells, displacing them. The endophyte has been found in all prothallia I collected outdoors, at different sites distant one from another. It harms the prothallia so little... that I am tempted to place it among the commensals rather than among the parasites." The following year H. Bruchmann (1885) reviewed Treub's work, which in general was confirmed by his own observations. He adds data on root hairs (p. 310): "In all the preparations studied by me, most of the root hairs were traversed by one or more thin, hyaline hyphae, which also grew through the tip. Towards the base of the root hair they become more vigorous, forming branches and septa, as well as showing a series of irregular, lateral outgrowths and swellings." Bruchmann also describes apparent vesicles (p. 312): "... many cells... enclose relatively large, rounded cell bodies. These are met singly, and also in twos or threes of varying size, in a cell. These structures have a smooth, in some cases a conspicuously thick and layered, distended membrane." He later describes spiralled hyphae (pelotons?) and apparent arbuscules which fill the lumen of cells with "hyphal nests." He emphasizes that colonized cells seem not in the least damaged, but he does not speculate on the possible relationships between fungus and host.

Bruchmann's report (1885) is the last of the prehistory of mycorrhizae, because the next to appear was Frank's (1885) first paper, in which the term "mycorrhiza" is proposed. That paper and its successors are subjects of the history rather than the prehistory of the subject, and thus are treated in other chapters of these proceedings.

BRIEF CHRONOLOGIES BY MYCORRHIZA TYPE

All papers quoted in our prehistory are arranged below in chronological sequence for each mycorrhiza type. We reemphasize that we have included only those papers which represent significant advances in knowledge and understanding of mycorrhizae. Many other papers which could be cited from this era either added little that was new or, in several cases, actually detracted from knowledge by erroneous observations or interpretations.

BRYOPHYTE ENDOPHYTES: Schacht (1854) establishes the fungal nature of the endophytes.

ECTOMYCORRHIZAE: T. Hartig (1840) describes the mantle and the subsequently termed "Hartig net" but does not recognize their fungal nature. Tulasne & Tulasne (1841) regard roots colonized with Elaphomyces mycelium to be parasitized. Vittadini (1842) emphatically states that the rootlets colonized by Elaphomyces are nourished by the fungus. Gasparinni (1856) notes "filaments" on the surface of much branched chestnut rootlets and the concomitant absence of root hairs. Bruchmann (1874) discovers that the Hartig net and mantle are both fungal. Boudier (1876) and Reess (1880) describe ectomycorrhizae in detail and consider them a pathogenic condition. Gibelli (1883) describes and beautifully illustrates ectomycorrhizae in detail and reports them on several genera of trees.

MONOTROPOID MYCORRHIZAE: Unger (1840) describes Monotropoid mycorrhizae and illustrates the mantle but does not recognize its fungal nature; he suggests that Monotropa is nourished by exudates from roots of associated trees. Lees (1841) states that Monotropa roots appear to be enveloped with fungus but then decides it is instead part of the root itself; he suggests that these "hairy fibres" take nutrients from tree roots and translocate them to the Monotropa. Rylands (1842) corrects Lees and demonstrates that fungi are actually involved. Kamienski (1881, 1882) provides the first accurate, detailed account of the mycorrhizae, asserts that the Monotropa is nourished by the fungi, and more or less suggests epiparasitism of tree roots by Monotropa via shared mycelia.

ORCHID MYCORRHIZAE: Reissek (1847) describes orchid endophytes, establishes that they occur in many orchid genera and species, and is the first to recognize them as fungi; he also attempts growing the fungi by a maceration technique. Schacht (1850) establishes that the fungi enter rhizomes from the outside and are not spontaneously generated within the cells. Prillieux (1856) demonstrates the connection of endophytes to external hyphae and observes that host cells are not harmed by colonization. Reinke (1873) describes passage of hyphae from one cell to the next and agrees that the host shows no sign of damage. Drude (1873) believes the endophyte to be a parasite. Pfeffer (1877) proposes mutualistic symbiosis for orchid mycorrhizae. Mollberg (1884) describes orchid mycorrhizae in new detail and establishes that the fungi can be Basidiomycetes in some cases.

VESICULAR-ARBUSCULAR MYCORRHIZAE: Nägeli (1842) describes hyphae and apparent arbuscules in Iris spp. roots. Holle (1875) reports endophytes in ferns and considers tham a degenerative influence. Mollberg (1884) records apparent VAM on Liliacee. Treub (1884) and Bruchmann (1885) describe VAM in ferns; both note that the colonization seems to cause little or no harm to the host.

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THE VEGETATIVE ORGANS OF MONOTROPA HYPOPITYS L.

by

F. Kamienski

(Translated by S. M. Berch from Memoires Societe Nationale des Sciences Naturelles et Mathematiques de Cherbourg 24:5-40, 1882)

Two years ago, I had the occasion to come across in the area of Leopol, on rolling land covered by forest, Monotropa hypopitys L., a plant that, by its distinctive appearance and certainly by its lack of chlorophyll, has for a long while attracted the attention of botanists. One also encounters frequently in the botanical literature not only observations on the structure and life of this plant, but one finds there as well special treatments dedicated to this subject. At the same time, the results given by the authors of these different works are so divergent, that it is absolutely impossible to conclude anything positive from them about the structure, development or the means of living (nutrition) of this plant. I therefore siezed on this occasion to carefully study and verify the assertions of my predecessors, ultimately to distinguish those that are true from those that are not. And since the results of observations on the vegetative organs of Monotropa are the most contradictory, I will at present be most concerned with them.

I believe it is unnecessary to enumerate here what has been written on this question, since on the one hand this has recently been summarized by Mr. O. Drude (6), and on the other hand, I will have the occasion in the following, to cite the different papers while submitting the results obtained by my predecessors to a conscientious critique.

Monotropa hypopitys L. is found in forests of conifers or other trees, especially in forests of fir, very close to the base of the trunk of these trees or a short distance from them. Before, one distinguished two species: Mr. Drude is of this opinion and defines them in the following way:

Monotropa glabra Roth. Sepala lanceolata, petalis breviter calcaratis late lanceolatis dimidio breviora; stamina biserialia aequilonga stigma glabrum attingentia; ovaria subglobosa tumida stylo brevi quadruplo longiora.

Monotropa hirsuta Roth. Sepala lanceolata petalis longius calcaratis spatulatis basi cuneatis paulo breviora; stamina biserialia, serie inferiore breviore, omnia stylo breviora; ovaria elliptica in stylum duplo fere longiorem attenuata. Perianthum pilosum, stamina pilis patentibus hirta, ovaria cum stylo pilosa, stigmatis margo inferior pilis densis patentibus ciliatus.

Other more recent authors, for example Mr. Ascherson (1) and Garcke (8), considered these two forms as simple variations of a single species. Besides, Mr. Drude contradicts certain authors, since he notes Monotropa glabra especially in conifer forests (spruce) and M. hirusuta in fir forests, while other botanists indicate the contrary.

The plants that I observed come from three different areas: 1) forest of fir and conifers in the region of Leopol, 2) similar forests situated on the Pieniny (on the banks of the Dunajec river), and finally 3) pine forests in the region of Varsovie. Everywhere I have noted that Monotropa has transitional forms between the two varieties, as this pertains to form and size, and pubescence of petals, stamens andcarpels. I am therefore of the same opinion as Mr. Ascherson and Garcke, which is to say that two different species do not exist, but rather two insignificant varieties. Concerning the location of Monotropa, I must add, contrary to the opinion of Mr. Drude, that forms most similar to M. hirsuta are found most commonly in conifer forests, while M. glabra is found principally in fir forests.

In general the shoot of Monotropa appears in the month of July. Its height surpasses on occasion 2 decimeters, and it bears squamulous leaves and a fertile stalk terminated by an apical flower. The fruit capsules contain very many seeds, brown in color, very fine and simple in structure. Figure 2 represents a seed with a testa composed of a small number of cells that covers the endosperm which arises from the central cells of the divided embryonic sac. The endosperm and the mature embryo appear as a united whole and can be easily taken for the embryo itself. This is the error commited by Mr. Charles Müller (13), who nonetheless gave a detailed description and very exact sketches of the development of this supposed embryo. It is only Mr. Hofmeister (9) who was the first to establish the distinction between the cells of the embryo and those of the endosperm, and these observations lead to the realization that the embryo is made up of 2 cells. Mr. the Count of Solms-Laubach (16) imagined that the embryo was composed of 5 cells, while Mr. L. Roch counted 9 of them, 8 of which would be formed by the terminal cell of the embryo, which as in Capsella bursa pastoris is divided by 2 longitudinal walls perpendicular to each other, first into 4 cells, which are cut off by another transverse wall perpendicular to the first two, which divides the embryo into 8 cells. The 9th cell comes from the "funicule" and forms the basal cell of the embryo, in other words, the hypophysis. In this way, according to Mr. Koch (12), the mature embryo of Monotropa would be analagous to the first stage of development of the embryo of dicotyledons.

I cannot agree absolutely with the affirmations of Mr. Koch mentioned above; I have observed frequently, while doing traverse sections of the endosperm and the embryo (as seen in Fig. 4, Plate I), only one of the 2 first divisions. It seems that the embryo does not always contain 9 cells and this number is often greatly reduced. In this way one understands why Mr. the Count of Solms-Laubach only found 5 of them. Besides, I must point out that the cells of the embryo, by

developing simultaneously with those of the endosperm, takes on, by means of this mutual pressure, polydedral forms and often change the positions that they first had.

The germination of seeds of Monotropa was described and illustrated for the first time by Mr. Chatin (5). He affirmed that the young Monotropa is a parasite that, by means of its fine, elongated base, pentrates the root tissue of the host plant and is topped off by the bud; that its conical base contains vessels which attain the form of liberolignous fascicles in the stem; and that in continuing to develop, it loses this basal part with which it previously had been in communication with the host plant thus abandoning it's parasitic role and subsequently drawing all of it's nutrients exclusively from the soil.

Mr. Drude arrived at a totally opposite conclusion. He observed young monotropas, germinating amoung the decaying needles of a fir forest, that had very fine roots without root caps and normally with branched, endogenous, fine roots. The shoot (P.10) thus, did not exist, nor did the haustoria, organs characteristic of a parasite. According to Mr. Drude, therefore, Monotropa is initially humicolous or saprophytic, becoming parasitic in time.

Despite all my efforts, I was unable to observe in a precise manner the germination of the seeds of Monotropa and thereby to examine the first stages of development of this plant. Seeds collected on various occasions were placed either in humus, in soil from around (bruyere), or in manure (in Van Tieghem cells which are used for the culture of fungi); but none of my results were conclusive. Through all of this, the proper conditions for the germination of Monotropa seeds remain elusive, and I am obliged to leave the solution of this question to the research of others; I abstain therfore from giving my opinion on the work of Mr. Chatin. As to the observations of Mr. Drude, to me these appear to be based on an error of observation. In fact, I have seen fine roots of trees that are so deformed by a parasitic fungus that they have the appearance of the roots of Monotropa with which they were so interlaced that one could easily mistake one for the other.

The vegetative organs of a mature Monotropa are observed to be highly branched roots. In sandy soil the roots are 3 decimeters deep while in humus soil they are but a few centimeters deep. The roots of Monotropa are distinguished by very slow elongation, and very abundant branching in all directions; they are so interlaced with the roots of other plants that they are very difficult to separate from them and this separation is rendered more difficult by the brittle nature of the Monotropa roots, which tend to break during preparation. On these roots adventitious buds develop and grow vertically out of the soil to terminate in an infloresence.

The root structure is as follows:

The tip of the root is represented by a longitudinal section in fig. 6 (Pl. I). It is distinguished by a poorly developed root cap, composed of numerous layers of cells, occasionally of only one. According to Mr. Drude, Monotropa hirsuta possesses a more developed root cap than does M. glabra; I have never noticed this difference, having often observed roots, on the same individual, with more or less abundant root caps. The epidermis is clearly distinguished from the periblem and possesses mother-cells in common with those of the root cap. In dividing, these cells produce external cells, which are those of the root cap, while those of the interior belong to the epidermis. The periblem and the plerome are not distinctly separated at the tip of the root, and it is only at a certain distance from the tip that their separtation begins to be evident. Therefore, this is the first known plant in which these two primary root tissues have a common origin, while its epidermis would be well delimited.

Beginning at the tip, one can easily observe the development of the three parts of which it is composed: the cells of the epidermis which are transfromed into a permanent tissue, with few changes during theri development. Root hairs are lacking; however one occasionally sees small protruberances on the cells of the epidermis (fig. 6, Pl. I), that seem to be rudimentary root hairs, but barely attain a height equal to half the width of the cells of the epidermis.

Particular attention must be given to the fungus, the mycelium of which covers the outer surface of the epidermis (Pl I, fig. 5, 6 & 8 g; Pl. II, fig. 2). The hyphae of this fungus are divided into cells by crosswalls; they are highly branched and form a very compact layer that is pseudoparenchymatous, often two or three times more thick than the epidermis itself (Pl. I, fig. 6). From the surface of this layer extend hyphae that are single or grouped into cords and stretch out into the surrounding soil. The fungus develops particularly at the apex of theroots at the point where the tissues are differentiating, while toward the tip of the root the layer of hyphae thins markedly, and over the root cap but a few isolated hypae grow. It elongates with the root and forms a type of glove around it. The extremities of the hypae that form this glove are intimately attached to the surface of the epidermal cells, penetrate below the cuticle and finish by pulling up some fragments of it. Other hyphae extend toward the exterior and grow at the very surface of the cuticle such that a large number of particles of cuticle are found at the interior of the hyphal layer and separate it into 2 layers (Pl. I, fig. 6). The fungus in queston is only found at the surface of the epidermis, never between living cells, but occasionally though very rarely and only in the most aged parts of the root, the fungus penetrates between the cells of the epidermis which are filled with brown contents (tannic) and are dead (Pl. I, fig. 8 g). I have never seen the hyphae penetrate deeper into the root tissue, as happens in other plants when their

roots are parasitized. I conclude from this that the fungus in question does not draw it's nutrition from Monotropa and is not harmful to it; but it fixes itself on the roots as an appropriate base for it's development. A series of numerous observations on my part support the conclusion that the existance of this fungus on the roots of Monotropa is the normal case, since I have never encountered a root that did not possess this hyphal glove.

I have not been able to date to determine the species of this fungus. The hyphae grown on liquid medium develop only to a certain point, then die without having produced spores. Mr. Drude advances, without presenting any proof, that this fungus is in the root cells of certain orchids (Neottia nidusavis, Goodyera, Corallorhiza, etc.). It has, however, escaped his attention that the mycelium of the fungus thatcan be found in some cells of the roots of Neottia is composed of hyphae that are thinner and have a very fine wall. As well, one knows that similar appearance of mycelium is no proof of identity, since mycelium of very different species can bear strong resemblance to each other. I would suggest, rather, without being able to confirm it, that the fungus that grows on Monotropa is the same as that which lives as a parasite on the extremities of the roots of conifers and other trees. This fungus deforms their roots and brings about their dichotomy. I have found, in fact, among the roots of Monotropa, a large quantity of other very fine, deformed roots belonging to trees that grow in their vicinity; they were so interlaced that the mycelium that covered them touched and even intermixed.

Mr. H. Bruchmann (4) describes in great detail the roots of Pinus silvestris which are deformed in this manner, without stating which species of fungus causes this deformation. Mr. Reess (14) has demonstrated that this fungus is Elaphomyces granulatus, of which he has collected the fructifications in great number among the roots of pines. As for me, I have never encountered this fungus around or among the roots of Monotropa, which is why it seems probable that we are in the presence not of a single, but of many species of fungus of which the mycelia are very similar.

On older parts of the roots of Monotropa the epidermal cells become disorganized at the same time as the mycelium that envelopes them, exposing the outer surface of the cortex the cell of which have become inert.

(At this point, approximately 13 pages of original text have been omitted since they deal in detail with structure and anatomy of the root system, shoots and leaves, with no mention of root-fungus associations.)

In the present state of science concerned with the nutrition of plants, we know that certain plants, that is to say those with chlorophyll, are capable of absorbing carbonic acid (CO^2) directly from the atmosphere and of decomposing it in the presence of light by giving off oxygen and absorbing the carbon for the production of the organic materials of which they are composed. This chemical process is called assimilation. Other plants, among which is found Monotropa, are lacking in chlorophyll and consequently not capable of such assimilation. They are therefore forced to absorb their carbon source in the form of an ogranic liquid by diffusion, and not to take it from the atmosphere. Up to the present, only two modes of nutrient absorption are known to occur in these plants. Certain of them gain nutrients by means of particular organs known as haustoria, which enter into the organs of other plants and extract from them more or less prepared substances, thus living at their expense; these are the parasites. The others attach themselves to soil rich in debris, particularly from plants, named humus, from which they absorb organic substance in solution through their roots; these plants are known as humicolous or saprophytes.

To which of these two catergories does Monotropa belong?

The first to have fixed his attention and his researches on the mode of nutrition of Monotropa was Unger (18) who classed it in his seventh order of parasites; and despite not having ever found in the thick and intricate skein of roots of Monotropa and fir (Pinus abies L.) either haustoria or other evident organ of junction, he claims nonetheless that Monotropa must be a parasite, because experience has shown that it dies at the same time as the roots of the tree on which it had survived. According to him, the existence of Monotropa depends on nutrients delivered by the roots of trees and absorbed by Monotropa. Similarly, Brandt (3) placed Monotropa in the same order of parasites as Orobanchia. W. Hooker (10), speaking of one species (Monotropa uniflora), puts in doubt the parasitism of this plant, because it can be raised independently of a host plant from seeds sown in soil rich in humus. Ducharte (7) also supports the idea that Monotropa is not a parasite. But much more explicit is Schacht's (15) confirmation, based on a special study of the vegetative organs of this plant, that Monotropa is not a parasite, or at least when a completely developed plant it has no remaining junction with a host. According to Schacht (17), Monotropa as well as certain Orchids (Epipogium, Corallorhiza, Neottia, etc) is nourished by the degradation products of certain plants which explain why it always appears close to these plants. To Chatin (5), as I've already indicated, Monotropa germinates as a parasite, but in it's subsequent development the plant ceases to be parasitic and lives in the manner of a humicolous plant. Finally, the Count of Solms-Laubach confirms the observation of those who, not having found the haustoria that characterize parasites, have excluded Monotropa from their ranks. Mr. Drude, in his oft-cited work as well as in his treatise on the morphology of the phanerogams which appeared in the Encyclopedia of Natural Sciences, approves only of a part of the opinions of these authors, saying that the variety Monotropa glabra is a

parasite that introduces it's fine roots into the roots of beech and pines from which it draws it's nutrients. The author gives a detailed description of these fine roots, particularly of those that adhere to the roots of pines. He calls them "parasitic junctions" and illustrates them on his plate IV, fig. 46, from above and in section.

Despite the most assiduous search I have found in my washes and root preparations of Monotropa, neither haustoria nor parasitic junctions nor any other similar organ, and I believe that the parasitic junctions of Mr. Drude are due to an error of observation. Even more convincing, I myself initially errored in taking abnormally developed roots of neighbouring trees for those of Monotropa. Similar roots had already been observed on various trees by Mr. Janczewski (11), Bruchmann and Boudiers (2), and these roots differ greatly from normal roots. They are all infested by a fungus that covers them in a thick and homogenous mycelium the hyphae of which penetrate between the cells of the epidermis and the cortex. The cells of these tissues are separated by a single layer of hyphae which branch densely in a single plane and can be seen on the surface of cell in tangential cuts of the roots. This fungus does not penetrate very deeply, notably not into the vascular cylinder. In this way, the structure of the infected roots and their external structure varies enormously. The cells of the cortex become larger and consequently less numerous. The root cap does not develop at all, or very incompletely, while the branching of the roots becomes more frequent. In the conifers, particularly in pines, these transformations are on such a large scale that normal branching is replaced by typical, true dichotomy described by Mr. Bruchmann, exactly as occurs in the Lycopodiaceae. For the rest, the hardened consistency of the roots, their semi-transparence and their pale colour all resemble the situation in Monotropa.

The roots of certain trees, particulary beech (Pl. III, fig. 7), have roots that greatly resemble those of Monotropa. They are short and abundantly branched, most frequently in a single plane. It is perfectly possible to distinguish them from the roots of Monotropa especially by the structure of the mycelium which in Monotropa does not cover the root cap and does not introduce it's hyphae into the root tissue. Going from the deformed apex toward the base of the root, one notices that as the mycelium disappears so the root changes it's structure to another more normal appearence. In fig. 7 (Pl. III) the fine roots a) and b) which are infested by the fungus are large and irregular; towards the base they become thinner and approach an even more normal state. The base of infested roots are most similar to those of Monotropa, as can be seen in the same fig. 7, near c) and d). The limit between these roots and branchings of larger roots is very clear, so much have they the appearance of a alien entity fixed to the beach root. Might not such roots with their broken apices be those self same "parasitic junctions" of Mr. Drude? But after more detailed examination of undamaged roots, it is possible to recognize that they are nothing but branches of the roots on which they are fixed. As well, anatomical structure demonstrates the uniformity of the tissues. The differences that Mr. Drude noted between the two, that is the absence of tracheids with areolate perforations in the so-called "parasitic junctions" - do not really exist, because these tracheids so characteristic of pine are only found in old roots and not in the young branches.

From all of the preceeding one concludes that Monotropa is not a parasite. Therefore it belongs to the second category of chlorophyllous plants, that is to say, to humicolous plants that draw their nutrients from the soil by the intermediary of roots following the law of diffusion. In this process, the epidermal cells of the root play the most important role. If Monotropa is one of the humicolous plants, let us examine how its process of absorption of nutrients from the soil occurs.

It follows from the preceding description of the structure of Monotropa that all of the most active parts of the root, where the most lively exchange of nutritive substances goes on in the epidermal cells and the interior tissue, all of these parts are covered by a thick and dense layer of mycelium which does not permit the roots a direct contact with the soil. The only parts of the roots that directly touch the soil are a few dead root cap cells, as well as the outer layers of the cortex of aged roots which are also composed of dead cells. But both of these, being dead, are incapable of absorbing nutrients. Consequently no other route by which the nutrient solutions might pass into and arrive at the root of Monotropa exists but that of the mycelium. This last is composed of vegetative hyphae of which those that are closest to the epidermis are so closely aligned with the cells that diffusion between them becomes not only possible but absolutely exists. Monotropa must therefore draw its nutrition through the intermediary of the fungus.

We are therefore in the presence of two vegetative organisms: on one hand there is Monotropa and on the other, a fungus, thus far unidentified, which help each other by living together. The roots of Monotropa offer to the fungus a convenient base by providing a larger surface and a stronger support from which to spread out and survive than would grains of sand or bits of soil, because I believe that I have sufficiently demonstrated that this fungus is not parasitic on Monotropa. In turn, the fungus returns the hospitality received by furnishing nutrients to Monotropa. The layer of mycelium replaces the epidermis and the hyphae proliferate out into the soil serving the physiological function of root hairs to Monotropa.

The nature of the fungus in question presents us with more doubts. It could be humicolous and nourish itself as do all other humicolous plants, including Monotropa, from the products of degradation of the organisms of the soil.

But it could also be a parasite, wherein a certain part of the mycelium lives at the expense of the roots of nearby roots of pines and beeches, and thus become identical to the previously mentioned parasite that lives on the roots of these plants. This latter opinion seems to me to offer more possibilities, not only because of observations already discussed dealing with the continuity of the mycelium on the roots of trees and that on Monotropa, but in addition because of the observation, which is beyond doubt, that Monotropa always becomes established in the proximity of the roots of these trees. In all cases, Monotropa and the fungus remain always in their reciprocal relationship; since, though there is a difference in the modes of nutrition between parasites and humicolous plants and though the material absorbed by the parasites that comes from living cells is more elaborate than that of the humicolous plants, nonetheless the food source of the humicolous plants as well as that of the parasites is a solution of organic substances, whether the fungus is humicolous or parasitic, the diffusion between its hypae and the cells of Monotropa will always occur among the various tissues of the same plant.

Doubtless many questions pose themselves here for our examination, and in particular the following: is this fungus absolutely necessary to the life of Monotropa? In other words, can Monotropa absorb with its epidermis and without the intermediary of the fungus? Because in this last case, one could pose an objection of fundamental importance, which is that the appearance of the fungus on the roots of Monotropa is purely accidental and has nothing to do with the nutrition of Monotropa in the manner of a humicolous plant.

But this question only appears to be fundamental, since, in as much as I have never seen roots of this plant free of all fungus, the opposite case would change nothing concerning the relationship between the fungus and Monotropa as it is above described. What occurs here is but an intermediary mode of nutrition observed in reality, without this having to be unique or absolute; similarly a parasitic fungus observed on whatever plant that feeds it, would lose none of its parasitic character if it can be raised artificially without the participation of the host plant.

This strange relationship between this fungus and Monotropa is not unique and isolated in nature. We can class it with other similar instances to which Mr. de Bary has given the name symbiosis. This is a sort of fusion or union of different organisms that, depending on their comportment one to the other, finish by accomodating to each other by acquiring different forms and changing their means of existing. In a few of these fusions one can see a battle for existence; the one attacks the other, takes from it its nourishment and usually brings about its death. These are the typical parasites, living on other organisms, such as many species of fungi, rust on wheat or Aecidium elatinum which causes spots on Abies pectinata DC., or even Cuscuta on clover, Orobanchia on hemp, etc. Other fusions are less offensive; the organisms unite without battle, so benefit in common from the same conditions for existence, or to live at the expense of each other but without prejudice, even to their mutual advantage. Of such fusions in the plant kingdom are Azolla and Anabaena, all epiphytes, certain hepatics and Nostoc, Utricularia nelumbifolia growing in the rosettes of the leaves of Tillandsia, and many others.

Mr. de Bary, agreeing with Mr. van Beneden (18), calls the first category of fusions antagonistic, and the others mutualistic. It is not necessary to prove that those two forms of symbiosis are but the extremes and that between them are to be found on infinite number of intermediary forms, as we can see in the parasites of diverse species.

To which category of symbiosis do Monotropa and its fungus belong?

The fungus is not a parasite of Monotropa (because it can be a parasite on the roots of trees), but it looks on these roots as a useful base and does no harm to Monotropa, which despite it grows well, flowers and produces seed. On the other side of it, though Monotropa draws its nutrition through the intermediary of the fungus, the fungus must not lose much as a result since in the contrary case it would not grow on its roots but rather on the earth that would do it no harm. Finally it is possible, even though this has never been verified, that Monotropa may be able to live without the fungus, as has already been suggested.

We see therefore that the symbiosis of Monotropa and its fungus cannot be counted within the first category, but definitely in the second; because not only do these two organisms not harm each other but just the opposite they mutually help each other. It is therefore this symbiosis that is the example of the most striking of the "mutualistic" union of two vegetative organisms.

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ON THE ROOT-SYMBIOSIS-DEPENDING NUTRITION THROUGH HYPOGEOUS FUNGI OF CERTAIN TREES.

by

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To promote the possibility of truffle cultivation in the Kingdom of Prussia, His Excellency, the Minister of Agriculture, Domains and Forestry, commissioned me to approach the matter systematically; I was to begin with scientific studies on the conditions of occurrence and development of these fungi. Certain facts had already been established by observations and through experience. For example, true truffles occur only with living trees, and in the Prussian truffle districts the investigations noted above especially established a relationship between truffle occurrence and particular tree species: beech, hornbeam and oak. Above all else was the union of Elaphomyces mycelium with pine roots, reminiscent of parasitism, as also recognized by Reess (Sitzungsber. d. physik.-med. Soc. zu Erlangen, 10 May 1880). From the outset these facts pointed the research to the question of whether the true truffles also establish a connection of the mycelium with living tree roots. As the following communication will show, this question must begin much farther back, because it presupposes knowledge about factors concerning the nature and nutrition of plants that science has not heretofore even slightly suspected. This shall be nearly the only topic of my present communication. It concerns the fact that CERTAIN TREE SPECIES, ABOVE ALL THE CUPULIFERAE, QUITE REGULARLY DO NOT NOURISH THEMSELVES INDEPENDENTLY IN THE SOIL BUT ESTABLISH A SYMBIOSIS WITH A FUNGAL MYCELIUM OVER THEIR ENTIRE ROOT SYSTEM; THIS MYCELIUM PERFORMS A WETNURSE FUNCTION AND TAKES OVER THE ENTIRE NOURISHING OF THE TREE FROM THE SOIL. Surprising though this proposition may sound, it can now be considered as solidly based through the scope of my researches.

When one examines feeder roots growing in the soil--the root system's last branches that represent the actual organs of nutrient uptake--of any of our native oaks, beech, hornbeam, hazel or chestnut, it becomes evident that they are generally composed of two heterogeneous elements: a core, which represents the actual tree root, and an organically united rind consisting of fungal hyphae. This fungal mantle completely encloses the root, even forming a continuous cover over the growing tip; it grows along with the root tip and behaves in every respect as an organically united, periferal tissue that belongs to the root. The entire structure is neither tree root nor fungus alone but resembles the lichen thallus, a union of two different beings into a single, morphological organ; it can be suitably designated as a "fungus-root" or "mycorhiza."

1. STRUCTURE OF THE MYCORHIZA. In surface view the fungus-root resembles the most of the fungal sclerotia in fine structure; it shows a pseudoparenchyma composed of irregularly and very tightly interwoven hyphae, the cells of which vary from about 0.0024 mm in cross sectional measurement to about 0.01 mm. in length (Table X, Fig. 3). The cell walls are relatively thin, sometimes nearly colorless and other times bright or dark brown, on account of which the mycorhiza appears bright or brownish or nearly black. The pseudoparenchyma is seldom one-layered, more often many layered and it then forms a rather thick mantle as seen in cross or longitudinal sections. This mantle does not merely lie intimately on the root epidermis; hyphae from it also penetrate between the epidermal cells of the root itself (Fig. 6). The cortex, including the epidermis, generally consists of about four cell layers. The epidermis and the subepidermal layer or only the former are composed of relatively broad, radially expanded cells, and as a rule only the epidermis is intergrown with hyphae. The inner cortical cells always remain free; I could never detect the hyphae in the endodermis or the fibrovascular bundles. These endophytic hyphae always grow only at the cell wall; they never enter the cell lumen but completely weave around all sides of most of the cells. These hyphae are only 0.0012 - 0.0024 mm in diameter, considerably thinner than those of the fungal mantle but clearly recognizable as a continuation of the mantle; the thin walls of the peripheral root cells restrict their growth to only a small diameter. Accordingly, one sees them most clearly in the surface view of the cell walls; because they are normally arranged in a tight, almost pseudoparenchymatous layer, they seem at first sight to be a delicate reticulation of the wall. Every cross section through the cell wall, however, demonstrates that this structure originates from the intercellular hyphae growing at the cell wall.

The surface of the mycorhiza is often perfectly smooth, so that the fungal mantle is sharply differentiated from its surroundings (Fig. 3). Formation of root hairs is rendered impossible by the tightly appressed fungal mantle and has never been observed by me. However, root hairs are often replaced by a similar structure of the fungal mantle: its superficial cells are extended as filaments which elongate by tip growth to spread among the surrounding soil particles. The manner in which this occurs varies greatly. Sometimes the the fungus-root is irregularly clad with a thick, loose felt of bright or pale brown filaments similar in diameter but varying between specimens from 0.0012 - 0.0036 mm in diameter. These meander back and forth and spread generally among the surrounding soil particles (Fig. 4), and one can frequently note that they are enlarged where they attach to small soil particles in the manner of lichen rhizines or root hairs of higher plants. Sometimes a multitude of filaments, longer and straighter growing, rather stronger, browner or more concolorous with the fungal mantle or blacker, radiate out into the soil, so that the fungus-roots seem to have a

broom or hair tuft at their tips when suspended in water. Sometimes the filaments coalesce here or there on the fungal mantle into multi-filament strands, which range in number of parallel filaments from a few to rhizomorphs almost as thick as the mycorhiza itself. Without precise examination, the latter are easily confused with the mycorhizas. The strands branch into branches of the same size or smaller and also anastomose. The hyphae of these rhizomorph-like structures, that commonly correspond to the color and size of the fungal filaments of the mycorhiza, often emanate from the surface into the surrounding soil as numerous individual filaments. In the truffle districts, especially in the neighborhood of a truffle growing in soil, they customarily abound in the soil, forming a system of innumerable branches and anastomoses spreading though the soil. Their connection with the mycorhizas of roots of any Fagales present is easily confirmed.

2. DEVELOPMENT OF THE MYCORHIZA. Longitudinal sections show us that the fungal mantle continues to the tip of the mycorhiza and completely encloses it, and that the core of the tip elongates through a clearly developed growing point, which bestows all characteristics of a true root in keeping with the nature of the tissues in question (Fig. 5). Therefore it must be assumed that the fungal mantle is capable of an expanding to keep pace with the elongation of the root which it encloses. Indeed, it has its growing region where the growing point and the zone of elongation of the mycorhiza core lie. The hyphae which comprise the part of the fungal mantle over the root tip are always much thinner than those at the zone farther back that is no longer growing, namely 0.0008 - 0.0024 mm broad and up to about 0.005 mm long; they change gradually into the larger cells farther back from the tip (Fig. 5). The fungal mantle thus enlarges itself by the continuing insertion of new filaments between the existing ones at the tip of the mycorhiza, and then the cells of the pseudoparenchyma thus constructed broaden to their final size. Both parts of the mycorhiza keep up with each other in this growth pattern, so that the fungal mantle always compactly overlies the growing root tip. Growth of fungal filaments into the epidermis is seen first where length growth has stopped, not in the growing region. Progressing from younger to older regions in longitudinal sections, one can can clearly follow the gradual penetration of the endophytic filaments from the surface of the epidermis. As to growth phenomena at the growing point of the root, we differentiate the common tissues of all root tips: apical meristem, procambium, protoderm, and root cap; and, we see how these meristems change to the common permanent tissues of the root: the central fibrovascular cylinder, the root cortex, and the epidermis. The classification of these meristems follows the types that apply to most dicotyledons as discussed in de Bary's Comparative Anatomy (page 12) under No. 3, i.e., where the root apex with a sharply differentiated apical meristem and procambium is covered by a common layer of initials for the protoderm and root cap (Fig. 5). The weak development of the root cap is remarkable: often at a given moment only a single cell layer is present, while as soon as the next one begins to split off from the protoderm, it again becomes disorganized. One often recognizes the remnants of the older cap layers as thin, brown masses, which are compressed by the surrounding fungal envelope and soon made indistinguishable. Understandable though reduced root cap formation may be in this case, in that the fungal mantle interferes with the space needed for root cap development, and as necessary as this reduction may seem because of replacement by the fungal mantle, it is still interesting to see that the inherent histological differentiation of the root remains intact despite the symbiosis.

How the mycorhiza develops with the young plant germinated in the soil is another question. Naturally, a fungal colonization of the radicle of the embryo in the seed is lacking. The tap root of the first stage of germination is also fungus-free. The tap root soon develops lateral roots, which remain rather slender and bedeck themselves along their length with numerous short, repeatedly branched feeder roots that almost appear coralloid. One can see the fungal colonization progressing on these lateral of second and lower order roots. At individual spots the fungal hyphae at first appress to the root epidermis for a ways, and, as they then develop branches that creep further over the root, they unite with themselves and with other branches. The fungal mantle is gradually constructed from such starting points. The fungal colonization seems to progress most rapidly on Carpinus; as a rule, the whole absorbing root system of one-year-old plants is converted to mycorhizas. It takes place relatively more slowly with Quercus, so that one can easily follow the process; one- and two-year-old plants or individual parts of the root system of older plants are often only partially colonized. These fungus-free feeder roots are than clad with root hairs as are the feeder roots of other plant species which always lack mycorhizas. Still, such fungus-free roots of Cupuliferae are relatively infrequent. And, at least the tips of such roots are often attended by fungi, while the mycelium spreads mainly towards the younger part of the root system and soon is able to grow around the tips of the young rootlets, because they grow slowly and always remain short. Only the stronger and very vigorously straight-growing roots, which penetrate root-free parts of the soil and which function as bearers of the actual feeder roots that develop there, frequently remain fungus-free. As with the young plants, the fungi colonize feeder roots on older parts of the root system directly from the soil.

CHANGES OF THE ROOT BY THE FUNGUS. The shape of the mycorhiza differs from the ordinary root not colonized by the fungus. When we cultivate other broadleaved trees as well as the Fagales under fungus-free conditions as will be mentioned below, the feeder roots are relatively thin and rather long, their lateral branches emerge monopodially at rather distant intervals,

and in shape and branching they resemble the parent root. In contrast, the mycorhiza shows a very slow length growth but attains a greater thickness, because the cell layers of the apical meristem and procambium proliferate somewhat more and the epidermal cells reach a greater width to form a short and relativly thick body. Moreover, a stronger tendency to branching develops, in that the lateral root branchlets occur at short intervals close behind the tip; these branchlets behave similarly to the parent rootlet in growth, form, and branching. The mycorhizas thereby form more or less coralloid growths (Fig. 1) that often develop into large, broad clusters (Fig. 2). The branching of the mycorhiza occurs by the endogenous mode common for roots, and the new growing tip that emerges from the parent rootlet therefore is clad with the parent rootlet's fungal mantle from the beginning. From then on the fungal mantle continues growth with the new rootlet branch as was described in this situation for the parent rootlet. The branching is strongly monopodial; in spite of the coralloid growth form, no dichotomy is to be seen. The first branchlet always forms behind the tip where length growth has ceased; branching proceed acropetally, so that the branchlet next to the tip is always the youngest and shortest. These branchlets occur rather clearly in longitudinal rows as is usual for roots: sometimes in 2, sometimes in 3, sometimes in 4 rows, occasionally in only one row, patterns that may partly depend on prevailing conditions of space. In addition, gradations occur in the changes of form produced by the fungus: sometimes the feeder roots approach the form of normal roots (Fig. 1a). Although in that case they are also enveloped by the typical fungal mantle, it is not formed as thickly as on roots which show the most pronounced coralloid form.

SUBSEQUENT FATE OF THE MYCORHIZA. The peculiar, combined organ of fungus and root as described above generally has a limited life span; still, it shares this property on the whole with feeder roots of woody plants. As the trees strengthen their root systems with age and invade new areas of soil, the feeder roots of the aging parts are lost, while new ones develop on growing parts of the roots in other areas of the soil. One finds that the mycorhizas, which have determinate growth, cease growing after a time, or regrow only on individual branchlets until they stop altogether and finally die off to shrivel and turn dark brown to black and brittle. For all of that, as already mentioned, they can form anew at other places, not seldom close to a deceased mycorhiza cluster. How long a mycorhiza remains alive may depend on a number of circumstances and may vary much; it may often persist for many years. Not infrequently one finds huge nests of fungus-roots which, considering their slow growth rate, must have taken a long time to form. As with normal tree roots in advancing age, we observe browning of the cells of the cortex in the oldest parts of a mycorhiza as the beginning process of dying, while the fibrovascular strand continues to function under the protection of the epidermis. Death of the fungal mantle goes hand in hand with that. In this same way those stronger growing tips of the mycorhiza destined to strengthen the perennial, lignified branches of the root system through further lengthening and thickening by establishing a cork cambium underneath the endodermis and a vascular cambium in the fibrovascular cylinder, naturally lose their fungal envelope. The fungal mantle is only for the younger root parts, particularly those concerned only with nutrient uptake.

REGULAR PRESENCE OF THE FUNGUS IN ALL AGES AND ON ALL ROOTS OF THE TREE. To study the roots of the Cupuliferae at various ages, I acquired one-, two-, and three-year-old plants of oak, beech, hornbeam, and hazel from various localities, as well as root samples with feeder roots of older trees, specifically a 120-year-old oak, a 120-year-old beech, a 100-year-old hornbeam, and a 40-year-old hazel. Throughout their lives, the feeder roots of these trees at all ages were developed as mycorhizas accompanied by the fungus. For the older plants it was also interesting how the fungus behaved in the various soil depths in which the roots occurred. I was able to pursue that especially for beech and hornbeam. In the uppermost soil layer, about 5 cm thick and relatively humus-rich, the tree roots customarily form the largest amount of feeder roots, and these, as already mentioned, are always developed as mycorhizas by the Fagales. These mycorhizas are surprisingly abundant in this soil layer, especially in truffle sites, so that the ripe truffles rest on and in a thick weft of mycorhizas. In deep soil, one can follow how the frequency of feeder roots decreases with depth, at first gradually but then more and more so. The stronger roots freely penetrate deep layers, but there they form feeder roots only sparingly or do so more only on branches that have penetrated upward into more shallow soil layers. In forest soil with the parent material at a depth of a half meter, roots could be followed into that material but at that depth form feeder roots only sparingly. Even there, however, the feeder roots develop as mycorhizas. One could explain this by a distribution of the root-colonizing fungus in all soil layers. More simply, however, it can be clarified by penetration of the parasite into the soil along with the root, which is always colonized as it elongates into the deeper soil layers.

OCCURRENCE OF THE ROOT FUNGUS ON PLANT SPECIES. It is extremely interesting that this soil-inhabiting fungus strictly selects the roots that it colonizes by species and thus abides by a strong systematic restriction. For example, when one studies the soil from a beech stand, one finds only the beech roots developed as mycorhizas. The entire herbaceous vegetation that occurs there, such as Oxalis acetosella, Mercurialis perennis, Anemone nemorosa, Asperula odorata, Viola canina, Convallaria multiflora, etc., as well as other woody plants, e.g., Hedera helix, Acer pseudoplatanus, have roots completely fungus-free and with root hairs, as is usual for plant roots. This is even the case when those roots are close by or growing through

a mycorhiza cluster. Now, to accurately determine the spectrum of host plants used by the root fungi, I examined most of our native woody plant genera in that respect and can first of all specify those that lack the root fungi: Betula alba, Alnus incana, Ulmus campestris, Morus alba, Platanus occidentalis, Juglans regia, Pyrus malus, Sorbus aucuparia, Crataegus oxyacantha, Prunus padus, Robinia pseudacacia, Tilia europaea, Acer platanoides and pseudoplatanus, Rhamnus cathartica, Cornus mas, Fraxinus excelsior, Syringa vulgaris, Sambucus nigra. Thus the great majority of plant families to which the native trees belong are free of the root fungus, as judged by the representatives here examined. Because the limitation to a small spectrum always points to the Cupuliferae, I have studied its most important representatives in this regard and have verified the presence of root fungi without exception. The roots of the following trees show an essentially constant fungal colonization of the kind described above: Carpinus betulus, Corylus avellana, Fagus sylvatica, Quercus pedunculata and sessiliflora, Castanea vesca from examples from the Rheinland as well as the Berlin Botanical Garden, and the American Quercus rubra from the local botanical garden. According to those results, one may assert that the root fungi are a special feature of all the Cupuliferae. Indeed, this symbiosis is so constant for this plant family that one could almost be tempted to count it as a systematic criterion. At any rate it is worth mentioning that the inclusion of the Betulaceae in the Cupuliferae, as accepted by the newer taxonomists, does not seem be be supported when one judges by the occurrence of the root fungi. On the other hand, it is also interesting to see a hint, through the occurrence of these fungi beyond the Cupuliferae, of a certain kinship of some other families with the Cupuliferae: the Salicaceae and the Coniferae. I have also found mycorhizas with them, but not so generally as with the Cupuliferae. Salix viminalis, caprea and aurita, as well as Populus tremula, originating from many sites, were colonized by the fungi in varying degrees, although no colonization was evident in other sites. I found roots of Taxus baccata, Juniperus communis and Larix europaea in the vicinity of Berlin to be free of the fungi, and those of pine, spruce, white fir near Berlin to be mostly but not everywhere colonized in the typical manner. Reess (loc. cit.) has similarly described this colonization on pine roots in sites where Elaphomyces occurs, but it is evident that the root fungus is much more broadly distributed on conifers that Reess believed, including sites where no fructifications of Elaphomyces have been found.

GEOGRAPHIC DISTRIBUTION OF THE ROOT FUNGI OF CUPULIFERAE. Once I had found the fungi under consideration on Cupuliferae in sites that produce no truffles, and the occurrence of those fungi seemed to be more and more a general phenomenon, it was appropriate to undertake a systematic clarification of the distribution question. By arrangement with His Excellency, the Minister, I have recieved for study roots of all species of Cupuliferae occurring in a large number of forest districts, representing as much as possible various soil conditions and geographical situations of the Kingdom of Prussia. These were mostly 1- to 3-year old plants, but samples of roots of older trees were also included. The primary result can be mentioned at the outset: THE MYCORHIZAS OCCURRED IN ALL REGIONS, AND NO CUPULIFERAE FREE OF ROOT FUNGI WERE TO BE FOUND. Beech, hornbeam and oak collected from various compartments of the forest district at our southwesternmost border near Saarbrücken were colonized by the fungi without exception, as were beech from Rügen Island, hornbeam from the Brödlauken Forest District in the Gumbinnen Administrative District at our eastern border, and indeed from all the regions lying in between from which Cupuliferae were examined. The results show that the differing elevations and soil types do not limit the fungus: it can be found in the flood plains, e.g., in the flood zone of the Elster in Schkeuditz Forest District and in the Elbe lowlands near Gräfenhainchen in Merseburg Administrative District. It occurs outside of flood plains as well in completely flat areas or gently rolling hills, e.g., it was constant on plants from the forest complex of the Dübener heath between the Elbe and Mulde, south of Wittenberg, from the Zöckleritz Forest District near Bitterfeld, from near Berlin, as well as from the Jülich flatlands. In hill and mountain regions the fungus was found at all elevations and exposures, on the plateus as well as in the vallies and hollows and on slopes, equally on north, south, west and east aspects, and without difference between gentle and steep slopes. The fungus also ascends with the beech into the higher mountain regions. Considering soil conditions, both flat and deep soil are suitable for the fungus; its behaviour here has been discussed above. Also, none of the geologic conditions of the soil exclude the parasite. It is constant in diluvial soil from various regions, indeed in strongly humic river loam no less than in light, more-or-less humus-poor sand (e.g., from various places close to Berlin), as well as in the intermediate formations of sandy loam and loamy sand with varying humus contents. Further, it is in the loamy sand soil that is the decomposition product both of carbonaceous sandstone (e.g. from Münster) and of the New Red sandstone (e.g. from Saarbrücken); in greywacke soil, e.g. from the Eifel, Westerwald, etc.; then in the red loam soil derived from the Rotliegendes sedimentaries from Sangerhausen. Finally, it occurs on all types of limestones, namely shell limestone (e.g., Freiburg on the coast, Heldrungen, Wanfried on the Werra, Friedland on the Leine), platy limestone (from southern Hannover, e.g., Alefeld, etc.), and no less on Eifel limestone (e.g., Schleiden in Eifel) than in chalk soils of Rügen Island. It merits mention that the fungus always seems to develop most luxuriantly on limestone substrates. Finally, it must be emphasized that no vegetation type hinders the appearance of the fungus on the Cupuliferae present; it occurs equally in high forests (of trees grown from seedlings), mixed high-coppice forests, and coppice forests, in fields, and no

less in places outside the forest where Cupuliferae are raised, such as parks, gardens, tree nurseries, etc. Indeed, I found the fungus growing along with the roots of plants that I had potted with soil in flower pots about 2 years before and then had allowed to grow.

With the root fungus of the Cupuliferae having such a wide distribution, it might seem odd that botanists have missed it before now. Plant roots, especially the root tips, have been studied botanically many times, but in general only seedling radicles have been used; when one selected the Cupuliferae, one thus had roots in a developmental stage that preceeds appearance of the fungus. Observers dealing with diseases of the Cupuliferae caused by root system disorders could hardly miss it, however. Much contemporary plant pathological work is conducted uncritically; this innocent fungus was found accidently in pathology work and, although no studies were conducted on its significance, it was assumed and declared to be the cause of some anomalous growth phenomena. However, it is an inalienable part of every beech and oak tree and, as we will see, serves as an important wetnurse for their nutrition. This misinterpretation in fact happened with the ink disease of chestnut that occurs especially in upper and central Italy, so called because it commences as an initial blackening and general dying and rotting of the roots. In truth, the cause of this disease is not investigated at all. Gibelli (Nuovi studi sulla malattia del castagno detta dell'inchiostro. Bologna 1883), who has been much occupied with this disease, believes it to lie in fungi that appear on the rotting chestnut roots and that he would characterize as Torula exitiosa de Seyn., Diplodia castaneae Sacc., and Melanomma gibellianum Sacc. The evidence on the causal relationship of these fungi to the disease is lacking, so much the more so because these and similar fungus formations occur as decomposers on plant parts rotting on and in the soil after death by any cause. Gibelli also noted the true root fungus on living feeder roots of chestnut. His description leaves no doubt about its identity with our fungus: the coralloid, tuberculate, swollen roots with tips capped by a pseudoparenchymatic mycelial net and entangled with branched rhizomorphs, as illustrated in plates IV and V of his report. He was so biased towards the concept of a root injuring enemy, however, that he equated this root fungus with the fungi on rotting roots listed above as a cause of the disease under consideration. Gibelli did declare that he had found the characteristic root fungus to be general in Italy on roots of healthy chestnut trees as well as oaks, beech, hazels, and other Cupuliferae. But even these observations were not sufficient to bring him to another interpretaton, that the injurious parasite had already expanded to a wide distribution in Italy and that, although it causes the disease, the tree would suffer from its attack not as long as it was growing vigorously but rather only as it developed weakness for other reasons. No further confirmation that Gibelli's viewpoint is erroneous is needed beyond what has been said above and what is to be said below about the biological significance of the root fungus. For us, the interesting fact emerges that Italy is included in the general geographical distribution of the mycorhizas of the Cupuliferae. The biological discovery of mycorhizas of the Cupuliferae has also been a possibility in Germany. R. Hartig (Untersuchungen aus dem forstbotanischen Institut zu Munchen. I. Berlin 1880, p. 1 ff.) studied a root disease of 1 to 3-year old oaks in various sites, as reported under the title "The oak root killer, Rosellinia (Rhizoctonia) quercina." He regarded that fungus as the cause of the disease, which produces a massive rotting of the tap root and the lower parts of the stem in the field and also grows on the soil surface. This fungus does not correspond with our root fungus; R. Hartig did not confuse the two, because he did not examine the oak roots well enough to discover the root fungus.

Since the occurrence of the root fungus is so general and regular that no Cupuliferae can be found without it, I pondered on a way to artificially free the plant from its nurse and to force it to take up nutrients independently. I succeeded in this by water culture. One- and two-year-old plants were lifted from the soil in late winter and transferred with intact root systems colonized by the fungus, as first confirmed, into a nutrient solution composed of compounds common for water culture. After some weeks, before the buds burst, root formation began. One could notice that the mycorhizas already present grew no further, but very bright,new roots, easily differentiated from the previously formed, darkly colored ones, formed laterally at various places. This is the common phenomenon of land roots not developing further when placed in water but producing new roots that initiate in the water. The root fungus in its characteristic form indeed also passed over to these new roots, in part with formation of loosely mantling filaments that assumed a type of colorless water form. But, it could unquestionably no loger keep up with the root formation. The base of the new roots still showed the extended fungus mantle, but it seemed less distinct, thinner, and often so interrupted that wide stretches of the epidermis showed fungal mantle only at spots, while the remaining places were bare and thus prepared for root hair formation otherwise repressed by the fungal envelope. The tips of the new roots were partly free of the fungus. In accord with this was the finding with a three-year-old oak cultivated from germination in water culture and never in earth: its strongly developed root system was completely fungus free. From all facts related so far we must conclude that the fungi of the mycorhiza find thrive best on roots in soil, that they are generally distributed in vegetation-supporting soil, and that from such soil they colonize the roots of the Cupuliferae.

THE SPECIES QUESTION OF THE ROOT FUNGI. The systematic position of the fungi in question can be determined only through acquaintance with their fruiting bodies. The occurrence of the mycelium on the roots must necessarily direct

attention to the hypogeous fungi, above all the Tuberaceae and many Gasteromycetes. It could appear strange that the ubiquity of the root fungus mycelium is not accompanied by a similarly general occurrence of the fungal fruiting bodies. That can be for two reasons, however: first, with extremely attentive searching hypogeous fungi can often be found even where one does not expect them; second and foremost, the presence of the mycelium of a fungus is not necessarily accompanied by the appearance of its fruiting body at just any time. There are examples enough that the mycelium of a fungus that remains sterile year round can grow without forming fruiting bodies, and that the latter appear only when certain environmental conditions are met. Hence we are presented with the question, is it possible to determine the fungi by their mycelial characters alone? It has already been described above how the fungal filaments branching off from the mycorhiza into the soil certainly show many variations in form, thickness, color, and connections. More precise observations, however, soon lead to the conviction that these characteristics are not usable without elaboration for specific differentiation, in that their variations and change from one to another on the same mycorhiza; at least in part they represnt changes in form of one fungal mycelium. If one compares the mycorhiza of a truffle site with that of a site not bearing truffles, no sharp differences even in significant morphological characters are to be found; many times the differences exist primarily only quantitatively, in the biomass development of the mycorhizas and the prevalence of the mycelium in the soil, which reaches the highest degree in truffle sites. Accordingly, we can assume that the fungi, which produce truffles in many regions, are much more broadly distributed than are the truffles themselves; perhaps they are quite common plants and, in the sterile regions, their fruit body production is limited by lack of the proper environmental conditions. On the other hand, it is unwarrented to conclude without further study that the similarity of the mycelia means the fungi are everywhere the same. There are various species of Tuberaceae which, in keeping with the common rule for fungi that related species present no reliably differentiating mycelial characters, probably cannot be distinguished by their mycelium. However, here I have come to questions beyond the scope of this paper that should be left for a later occasion with results of still pending studies.

BIOLOGICAL AND PHYSIOLOGICAL SIGNIFICANCE OF THE MYCORHIZA. The organic union between the roots of Cupuliferae and the fungal mycelium into a morphologically independent organ, the intimate, reciprocal dependence following the growth of both parts, and the tight relations of physiological functions that must exist between the two--these relationships would seem to be a new example of symbiosis in the plant kingdom. It goes beyond the lower organisms to the most highly developed plant form, the trees, and is thus incontestably most unexpected and surprizing. First of all, the fungus mycelium must be considered as an undoubted parasite on the living cupulifer root, as is evident from the whole manner of its colonization and penetration into the growing root. As obtains for all parasitic fungi, the basic nutritional need of the fungus is primarly the carbon compounds procured from the photosynthesizing tree. In contrast, the fungus is evidently independent in regard to uptake of soil minerals, in that it alone is in contact with the soil by its periferal position on the mycorhiza and the innumerable filaments it extends into the soil to overgrow soil particles like root hairs. Now the question of great interest must be, is the tree damaged from the fungal parasitism of the roots? We know from a thousand cases that parasitic fungi damage their host plants. The morphological changes assumed by the tree roots under influence of the parasite undoubtedly can be characterized as hypertrophy or gall formation, albeit relatively weak. This suggests an irritation exercised by the fungus on root growth. However, the root is in no way killed by the fungus, and in spite of its change it does not loose the capacity of functioning for the tree, as the prosperity of the latter sufficiently proves. On the same basis, the idea of the fungus depriving the tree of mineral nutrients carries no weight. Were this so, healthy beech and oaks could actually exist, because each cupuliferous tree is accompanied by the fungus from its first year of life to advanced age. We conclude from all of that, that THE ROOT FUNGUS, AT LEAST IN THE MYCELIAL STATE, CAN INFLICT ABSOLUTELY NO DISADVANTAGE ON THE TREE. This fact presses the stamp of symbiosis on this relationship, because both of the united organisms live together in reciprocal assistance without harm to each other. The fungus fulfils a reciprocal service for what it receives from the plant, a service of eminent significance, for it represents the most important factor in the nutrition of the tree. It cannot be disputed that water and nutrients needed by the tree from the soil are supplied only through the mediation of the fungus, because it envelops the entire surface part of the feeder roots and its filaments play the role of root hairs of other plants in their intimate contacting of the soil components. In the enlargment of the volume of the epidermal cells of the root and in their complete envelopment with hyphae of the fungus, we must recognize an arrangement which is probably intended for the function of nutrient uptake by the tree. The fungus takes up soil minerals not only for its own nutriton but also for that of the tree, and we must therefore consider that THE ROOT FUNGUS IS THE SOLE ORGAN FOR UPTAKE OF WATER AND SOIL NUTRIENTS BY OAKS, BEECH, ETC.; it functions in respect to this nutrition as the wetnurse of the tree. So, the Cupuliferae show, in contrast to autotrophic plants and trees, a relationship that one can term "heterotrophy," that is, the nutrition from soil with help of another organism in a truly splendid scale, known heretofore only with lichen gonidia and some lower algae living within higher plants.

The symbiosis of the Cupuliferae most closely parallels mutatis mutandis the lichens,

specifically in its biological character, i.e., both requirements and outputs are found for the nourishment of both partners through this life association. Indeed, the root fungus is analogous to the lichen hyphae and the tree to the lichen gonidium; the comparison need not be elaborated further. A complete coincidence seems to prevail even relative to the question of how far these symbiotic relationships are necessary or dispensable for both parts. As is known for the lichens, the symbiosis is not necessary for existence of the gonidia, in that they can develop further in the free alga form after isolation from the lichen; similarly, as previously mentioned, the Cupuliferae can be cultivated fungus-free in water culture for years. Of course, the Cupuliferae free of fungi do not develop strongly in water culture. Still, that is at least partly attributable to the unusual medium, for it is manifested similarly in this culture method by other land plants. Whether the Cupuliferae can perhaps nourish themselves better with their fungus nurse than without is not known from these studies; the other standard for this question fails us: there seem to be no adult Cupuliferae free of root fungi. On the other hand, as the lichen hyphae do not develop thriftily and in any case never attain typical fruiting without the gonidia, so also seem the mycorhizal fungi to depend on the chlorophyllous tree for their development. So far I have not succeeded in establishing a further growth of the fungal filaments from pieces of living mycorhizas put in water or in fungal nutrient solutions such as plum decoction. Moreover, the strong dependence of the occurrence of truffle fruiting bodies on the presence of living trees is of emphatic significance here. This would not preclude the supposition in explanation of the general distribution of the fungus in plant-supporting soil, that a weak, perhaps somewhat saprobic, development of the fungus is possible in the soil without the nourishing tree. Finally, the root fungi are also reminiscent of the other conspicuous dependency of the lichens, the relation to substrate: as many lichens are restricted in their occurrence and their perfect development to quite specific types of rock, so also occur the fruiting bodies of hypogeous fungi, in remarkable relationship to the properties of the soil aside from their dependence on the nourishing trees,, e.g. the edible Tuber species point with certainty to an underlying limestone. The two symbiotic relationships here compared thus differ only morphologically through the differentiation and organization of the body of the phanerogam as compared to the alga.

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EXPLANATION OF THE FIGURES

Fig. 1. Specimen of a root of Carpinus betulus laterally clad with mycorhizas that are mostly coralloid-thickened; near "a" a branch system which is thinner but also colonized by fungi. Natural size.

Fig. 2. A lateral root with a cluster of mycorhizas coming off a taproot ("r") of a one-year-old Carpinus betulus. Natural size.

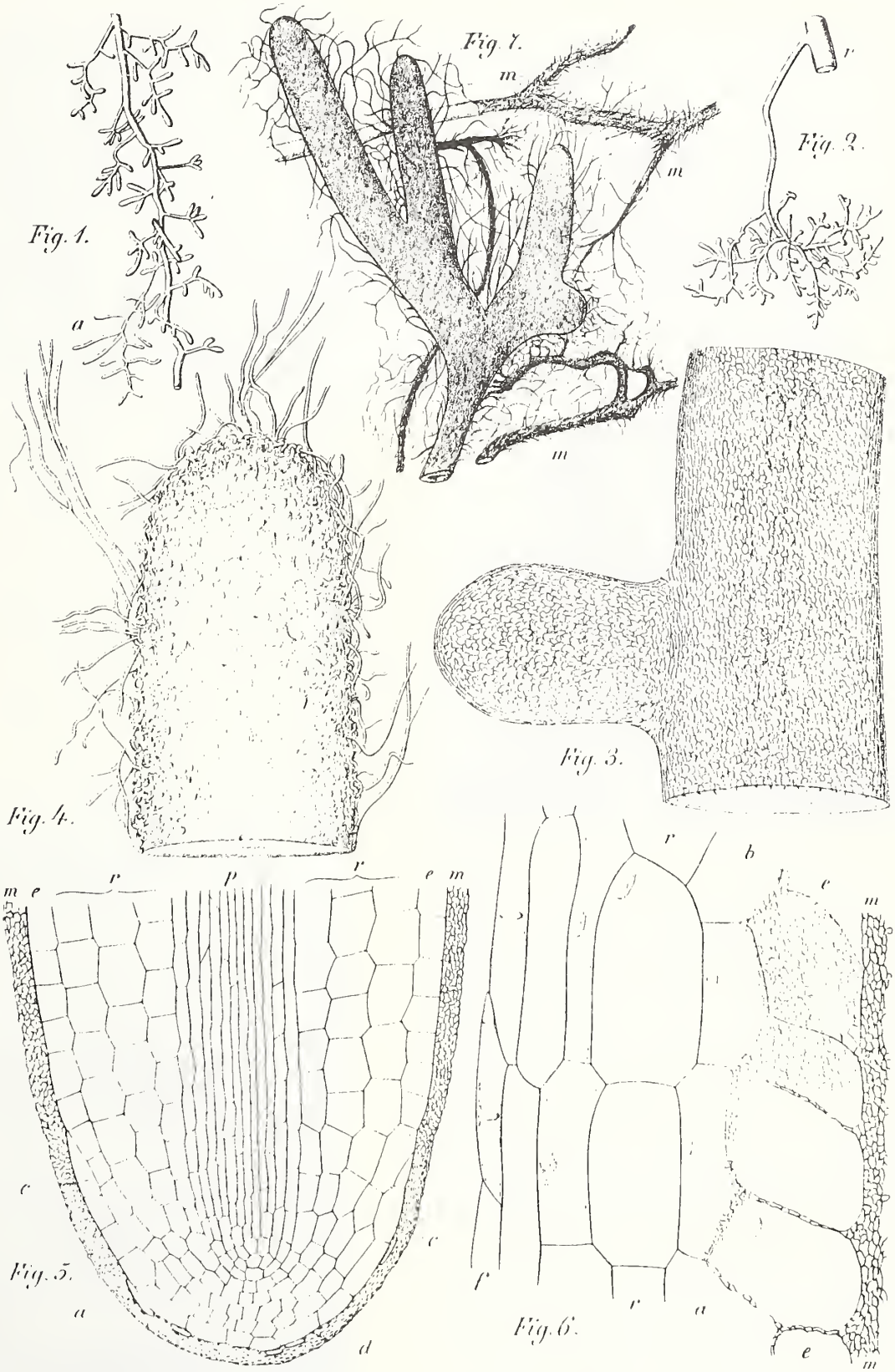
Fig. 3. Specimen of a mycorhiza of Carpinus betulus with a short lateral branch; the surface view illustrates the form of the outer smooth fungal envelope composed of a small celled pseudoparenchymatous tissue, through which one sporadically sees the outlined contours of the underlying cell net, the actual epidermis. x 145.

Fig. 4. Tip of a mycorhiza branch of Fagus sylvatica, showing the form of fungal envelope with loosely flocked, superficial hyphae that grow out into the soil in irregular courses. x 145.

Fig. 5. Longitudinal section through the growing tip of a mycorhiza of a one-year-old Carpinus betulus: "p" the procambium, "rr" the cortex, "ee" the epidermis, "cc" the ephemeral root cap layer which covers the crown of the root, "d" the general layer of initials of the protoderm and root cap as well as the epidermis "e", and from which also forms the root cap layer "c" through periodically repeated divisions to the outside; "mm" the fungal envelope, composed of pseudoparenchymatous tissue which surrounds the entire growing tip of the root, the cells of which are still much narrower at the tip, the youngest part of the mycorhiza, than those farther back; one sees the dead, detached root cap layer around the root tip as remnants of separated, once contiguous cells, e.g. lying near "a" and woven about with filaments of the fungal mantle. x 480.

Fig. 6. Part of a longitudinal section through an older portion of a mycorhiza of Carpinus betulus, leading through the epidermis "e," the cortex "r," and the cells that border the fibrovascular strand "f;" "a" the direction towards the root tip, "b" towards the root base, "m" the fungal envelope, which forms the surface of the mycorhiza and from which a pseudoparenchymatic cell layer continues inward to weave around the walls of the epidermal cells; in the lower part of the figure one sees the same in cross section of the epidermal cell wall, in the upper part a surface view, not unlike a fine, reticulate thickening of the wall. x 480.

Fig. 7. A mycorhiza of Fagus sylvatica from Alefeld in the vicinity of a truffle (Tuber aestivum), with a very strongly developed fungal envelope, which extends through the soil partly as free mycelial filaments and partly as almost rhizomorph-like mycelial strands that in turn disperse as free mycelial filaments. x 40.



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MYCORRHIZA: THE FIRST 65 YEARS; FROM THE TIME OF FRANK TILL 1950.

By

J.L. Harley

Keywords--

Introduction 1885-1900

One must realise the scientific background at the time that Frank (Fig 1) published. Agricultural research, giving an organised corpus of knowledge of plant nutrition, had been engendered especially by Leibig in Germany and by Lawes and Gilbert in England.

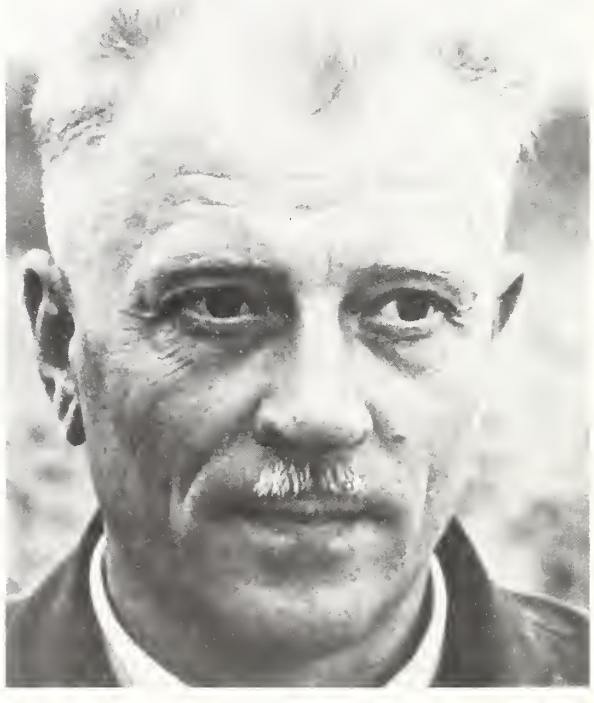
I.A.B. Frank

The importance of nitrogen to plant growth had been stressed and the departure of leguminous crops from the expected reliance on nitrogenous compounds had been confirmed. It was explained by Hellriegel and Wilfarth in 1886 (published 1887) and by Lawes and Gilbert two years later, followed in the same year by the isolation of Bacillus radicicola by Beijerinck. Associations between algae and fungi in the lichens had been confirmed by Schwendener in 1867; and in 1884 de Bary (see 1887) had introduced the term symbiosis. His contributions were extremely far-seeing, for he perceived clearly the gradation between saprophytes, necrotrophic parasites, biotrophic parasites and mutualistic symbionts that we hold today. It was well known that mushrooms, toadstools and truffles, tended to be associated with particular trees in natural habitats and hence, because of the economic importance of truffles research was financed to provide an explanation. Frank, plant physiologist and forester, embarked on his important researches because the German State Forestry Department wished to find out whether the production of truffles could be increased.

A.B. Frank and reactions to his views

The possibility was that the mycelium of truffles was parasitic on the roots of trees. Frank discovered a regular association of fungal tissue (the mantle) with the root tissues of the trees and described it emphasising its similarity to the lichen thallus. His paper in 1885 was, the first general account of mycorrhiza. He recognised that the infection was widespread amongst tree species, Fagus, Quercus, Carpinus, Castania, Betula, Salix, Tilia, Pinus, Abies, Picea etc - but he described woodland herbs as lacking mycorrhiza, because their roots lacked fungal mantle. Two years later Frank (1887) recognised that many, especially Orchidaceae and Ericaceae but also some trees eg. Fraxinus, Acer, Platanus and Taxus - were infected in a different way. He distinguished two kinds of mycorrhiza, ectotrophic and endotropic. Vesicular-arbuscular mycorrhiza, was described by Schlicht (1889), in many angiosperms.

Frank's work emphasised the following points. The rootlets were completely covered by a mantle of fungal tissue from which hyphae passed inwards between the cells and into the soil. The network in the cortex, now called the Hartig net, had been described earlier by Theodore Hartig in 1852, without appreciation that the strands were fungal. Frank described the infected roots as having coralloid form and branching, and devoid of root hairs. Trees became infected during their first year and remained so throughout life. There was variation of intensity of infection according to soil conditions, but in the humic layers of the forest they were heavily colonised. Frank (1894) showed that mycorrhizal infection was a beneficial, symbiosis between tree and fungus (see Figure 2).



L.B. T.Dominik

2. Growth of Pine (Frank 1894)

Many denied the widespread nature of mycorrhizal infection of trees. Robert Hartig (1886) said that, on oak in parks and gardens he found "no trace of mycorrhiza" and in summer when the trees were believed to absorb most water and salts, new roots without fungus were most frequent. The constancy and widespread nature of ectomycorrhiza was a problem that concerned workers following Frank's first publication. Most authors, whether they agreed or not with Frank's hypothesis of function, agreed that infection was of general occurence. The opposition, eventually came to agree also, but Hartig never subscribed to the view of beneficial function of symbiosis although van Tubeuf (1888, 1903) did.

At the same time surveys were being made of the occurence of types of mycorrhiza, and its widespread occurence in large numbers of pteridophytes, gymnosperms and angiosperms was recorded. Ectomycorrhizas were found to be restricted to conifers and dicotyledons, and to woody perennials.

After 1900 few denied that ectomycorrhizas were a normal in large numbers of woody plants. One such was W.B. McDougall who between 1914 and 1928 avowed that ectomycorrhizas were infrequent abonormal, pathological structures.

A second problem, related to the causes of variation of intensity of mycorrhiza infection. If reasons for its variation could be found, much would be learned of the functioning of mycorrhiza. Frank observed a correlation between the abundance of mycorrhizas and humus content of soil and in 1888 showed that in Fagus seedlings from meadow soil, garden soil, forest humus, sterilized or no, mycorrhizas were less abundant in soils of low humus content especially in the absence of forest humus, and also in sterilized humus.

The results were interpreted as showing that mycorrhizas are concerned in utilisation of compounds found in forest humus.

Theories of Function

Many observers have corroborated the high frequency of mycorrhizal development in humic soils although they would not now agree with the early explanations of it. One of the first was that the mycorrhizal fungi are found in greatest abundance and activity in humus because from it they draw nutriment and are enabled to attack, infect and injure the tree roots, (Robert Hartig, 1888, W.B. McDougall, 1914, Lange 1934).

Frank was convinced that the humus provided compounds which could be absorbed by the fungus and passed to its host. Although he also assumed that the main carbon supplies for the fungus came from its host. His main assumption, based on the absence of nitrates from acid humic soil, was that the fungus aided the uptake of nitrogenous subtances, ammonium and organic nitrogen, from the humus (Frank 1894) and increased the growth of the host (Fig. 2).

The nitrogen theory was widely accepted because of the circumstantial evidence provided and because of the preoccupation with nitrogen and plant growth and the view that plants were unable to use sources of nitrogen other than nitrate if uninfected.

The nitrogen theory engendered much experimentation up to the end of the period (1950), although alternative theory, the mineral salt theory, put forward by Stahl (1900) and modified later has been a competitor. The two are not mutually exclusive.

Stahl started from the standpoint that some species were uninfected and others were mycorrhizal. Following the hypothesis of his day, that the rate of absorption of ions from the soil is directly dependent upon the transpiration throughput, he estimated rates of water uptake and loss in mycorrhizal and nonmycorrhizal plants. Because of the magnitude and difficulty of the task of direct measurement, he resorted to

indirect methods, eg. anatomical observations, for estimating expected transpiration rates. Mycorrhizal infection was associated with small root systems and the absence of features which indicated rapid water loss, (eg. hydrathodes, many stomata etc.). The mycorrhizal hyphae were therefore believed to aid the absorption of all minerals. On the other hand, plants with extensive roots, large leaves, many stomata etc. had less mycorrhizal development and were able to absorb mineral salts readily into their roots. Mycorrhizal plants were able, by virtue of their fungal associates to compete with fungi in woodland soils for all mineral nutrients and it was not in the absorption of nitrogen compounds alone, but in that of all soil-derived nutrients, that mycorrhizal infection functioned.

In relating these ideas of Stahl to moden views, two points are apparent. First that the idea of competition between mycorrhizal fungi and soil fungi in humus is echoed in modern times. Secondly, the fungi (in Stahl's model), absorb from the soil irrespective of transpiration except in so far as the hyphal ramifications in the soil offset inactive transpiration.

From the turn of the century till the 1920s there was less interest in ectomycorrhizas, than in other kinds of mycorrhiza. However a number of separate lines of investigation can be traced throughout the twentieth century.

(1) The fungi of ectomycorrhizas and their physiology.

(2) Factors affecting intensity of mycorrhizal infection.

(3) The growth and development of the mycorrhizal root system.

(4) The affect of mycorrhizal infection on growth and nutrient absorption by the host.

The book of M.C. Rayner (1927) (Fig. 3) was an important milestone in this study for it was the first extensive summary of the subject of mycorrhiza.

Figure 3 M.C. Rayner.

The Fungi of Ectomycorrhizas

Although A.B. Frank set out to determine the factors affecting the production of fruit bodies of truffles he did not reach any conclusion. The association of Elaphomyces and Tuber with tree roots continued by others to be regarded as a form of parasitism. Woronin (1885) pointed out that he had made similar observations to those of Frank and concluded that the fungus concerned was a Boletus. Truffles did not occur in Finland where he worked. Over the ensuing years workers recorded the close relations of fruit bodies or traced the mycelium of fungi to mycorrhizal roots. Noak (1889) for example recorded the relationship of Geaster fimbriatus and G. fornicatus to Pinus and Abies, Tricholoma russula to Fagus and Pinus, Lactarius piperatus and Cortinarius species to Fagus and Quercus and so on. In the following years large numbers of Agaricaceae, Boletaceae and Gasteromycetes amongst the Basidiomycetes and many Ascomycetes are accepted as mycorrhizal. M.C. Rayner (1927) gives a list of authors who had then added to this list of fungi. Benjamino Peyronel (Fig. 4) from 1917 onwards wrote on the fungi associated with various kinds of mycorrhizas. He showed that particular species of fungi are not closely associated with one particular host, and that one host may form mycorrhiza with many species of fungi.

Figure 4. B. Peyronel.

Peyronel considered that mycorrhizas formed by different fungi might be readily recognisable. He concluded that the appearance of a mycorrhizal root was the property of the host, whereas the details of the structure, colour and ornamentation of the sheath, and the nature of the Hartig net were fungal. These observations were the first where ectomycorrhizal organs were classified into "species". Melin (1927) (Fig. 5) made such a classification of the types of infected root in pine in raw humus. A somewhat similar classification was made of mycorrhiza in Fagus by Harley (1937).

Figure 5 E. Melin.

Such classifications, become important when the properties of mycorrhizas, collected from the wild, are studied in experiments especially physiological experiments. However Dominik (1955) and later workers have studied the distribution of kinds of mycorrhiza classified in an almost analogous manner to lichens. (Fig 16).

From 1917 onwards Elias Melin published work on mycorrhizas which concerned the fungi of ectomycorrhiza. He was the first to isolate and back-inoculate fungi of ectomycorrhiza, to make isolations from fruit bodies which formed mycorrhiza in culture, and to examine the physiological properties of many in culture.

The ectomycorrhizal fungi, which grow slowly in culture, were found to be

(1) dependent on simple carbohydrates and

(2) prefer ammonium or organic nitrogen,

(3) require thiamine and sometime other vitamins

(4) are stimulated to grow by exudates of roots,

(5) are sensitive to compounds in humus and litter,

(6) are not resistant to competition

(7) have no known characteristic peculiarly theirs.

Factors affecting infection and its intensity

It was observed that the intensity of mycorrhizal infection appeared to be greatest in acid, raw humus soils and lowest in cultivated soil (eg. Sarauw 1893). This was ascribed to the distribution of the fungi in humic soils, and their probable absence cultivated soil; a contention which may have some substance, but is not a sufficient explanation. Stahl (1900) contended that intense mycorrhizal development was correlated with poverty of available nutrients and this seems upheld by Melin's (1917) observation that pines were heavily mycorrhizal in drained peat and less so in mull humus. Melin correlated the intense development with low nitrogen availability and low nitrification rate.

Figure 6 A.B. Hatch

Hatch (1937) (Fig. 6) using Pinus sylvestris grown in a range of humic soils found that the higher the nitrogen mobilization rate the higher the infection intensity. On the other hand in woodland soil, increased nutrient availability reduced infection. He resolved this dilema, showing that in very poor soil increased nutrients increased infection but in good soil greatly diminished infection. He concluded that within the starvation range, increased nutrition increased both growth and mycorrhizal development; but above the starvation level, low or unbalanced supply of nutrients (NPK) increased infection.

Björkman (1942) demonstrated that high light intensity increased the intensity of mycorrhizal infection.

Björkman's conclusion that all the nutrient factors and light operated through the same system, was an important driving force in research.

His conclusions fitted with the observations of Huberman (1940), Harley (1948), Warren Wilson (1951) that infection in seedlings takes place after the primary leaves are set. Much discussion led to analyses of root systems for carbohydrates using improved methods and agreement with his main thesis.

Although the early workers assumed that mycorrhizal fungi were active vegetationally in humic soils in the absence of their hosts, this became improbable in the face of the work of the Melin school on the carbohydrate physiology of the fungi. Again the regular association of fruit bodies of some species with trees even when growing in isolated positions suggested an obligate dependence in ecological conditions. In 1938 and 1939 L.G. Romell carried out trenching experiments which showed that many mycorrhizal fungi failed to fruit if isolated from active roots. The sporadic appearance of mycorrhizal infection in unexpected surroundings can be generally ascribed to the arrival of spores although as Fries (1941, 1943), showed, the spores have a low percentage germination in vitro. Infection explicable by spore infection is recorded by McComb (1943), Rayner and Nielson-Jones (1944) and others. The further work in the field of spore germination still proceeding is leading to important results.

The conclusions from this work on the fungi and infection were that the fungi are ecologically obligate biotrophs, depending on their host probably for carbon compounds and growth factors. The host seem to be equally ecologically obligate symbionts in soils of low or unbalanced nutrient supplying power.

The growth and development of the mycorrhizal root system

Ectomycorrhizal trees possess heterorhizic root systems, differentiated into long roots of potentially indefinite growth, and short roots of restricted growth in length. The short roots, are converted by infection into mycorrhizas, whereas the longer roots are usually incompletely infected or uninfected. The lack of recognition that most potentially mycorrhizal roots are predestined to remain short led early workers to believe that fungal infection leads to greatly retarded growth. The fact that the short roots which became mycorrhizal had a short life period was held to support the pathological view of R. Hartig, von Tubeuf and McDougall. Others pointed out that infection led to repeated branching, so that mycorrhizal root systems might compensate for reduction in growth in length, (eg. Stahl 1900 , Melin 1925). In 1930 Aldrich-Blake pointed out that there was a great size difference in protoxylem diameter between those lateral roots of pine which are short and those which become long roots. The short roots with small diameter protoxylem may become infected, dichotomize, and produce lateral mycorrhizal systems. Those that do not become infected soon abort.

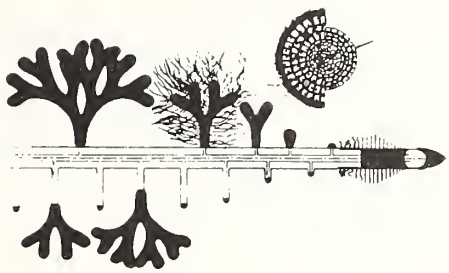


Figure 7 Effect of infection (Hatch 1937)

Hatch and Doak (1933) further reemphasized that short roots did not owe their characteristics to the effects of mycorrhizal fungi. In Pinus strobus they lacked a root cap, had a 4-layered cortex, slow elongation rate, and no secondary growth. After infection their rate of growth did not diminish and they dichotomized repeatedly so that one branch system might involve as many as 80 apices. In the uninfected condition they remained simple and short. The conclusions reached by Hatch (1937) from his work with Doak and that of others is summarised in the Fig. 7.

Figure 8 K.D. Doak

It was implicit from this work that the final state of the mycorrhizal roots in Pinus was due both to properties of the rootlet itself together with the effects of the fungus which engendered a continued growth and branching.

(3946)
Clowes (1949, 1950, 1951) carried out anatomical study of the root system of Fagus sylvatica, in which, like most trees other than Pinus, the roots and mycorrhizas do not branch dichotomously and the differentiation of long and short roots is not so extreme. He concluded that the architecture of mycorrhizal and non-mycorrhizal roots was similar but that in mycorrhizas the root cap was reduced and mature tissues of cortex, endodermis, and xylem differentiated closer to the apex. In addition the epidermal cells were elongated laterally and not axially. The epidermal and cortical cells were not "hypertrophied" as has been suggested, because their volume was reduced or unchanged. The diameter of the infected root was however increased, as Hatch and Doak observed in Pinus.

Slankis (1948, 1949) had just published two papers which showed that exudates of Boletus variegatus and dilute β-indole acetic acid solution had similar effects causing a dichotomy of excised rootlets of Pinus sylvestris in culture. Clowes realised that these results were in line with his work for when infection began the root surface was first colonised by an incipient sheath from which the Hartig net penetrated between cells already laterally elongated. J. Warren Wilson (1951) demonstrated with Fagus that many of histological characters of the mycorrhiza - size of root cap, size of meristem, proximity of maturation to the apex for instance - developed in the short roots in the total absence of fungi. (see Warren Wilson and Harley 1983).

In summary as Hatch and Doak (1933) and Hatch (1937) first seriously contended, infection increases the surface area of the infected roots

There are complications which arise from experiments comparing the effect of infection on root development eg. Hatch (1937), Mitchell (1937), Finn (1942). Mycorrhizal plants have smaller root/shoot ratios than uninfected plants. On the other hand McComb (1938) showed the total number of short roots per unit weight on mycorrhizal Pinus virginiana was similar. Half of them were mycorrhizal on infected plants, hence the surface area would be very much increased by their branching.

The effect of mycorrhizal infection on growth and nutrient absorption.

Frank's claims that mycorrhizal infection improved growth were not widely accepted at first. Many criticised his views. For instance Henschel (1887) examined the relation of intensity of infection to health of Picea excelsa seedlings and concluded that the degree of weakening increased as the mycorrhizal development increased. However through the years 1917 to 1950 researches indicated that infection increased growth and nutrient uptake. Melin (1917) had shown that in bog areas it was the mycorrhizal conifers that survived and that infected plants were extremely unthrify. The necessity of mycorrhizal infection for growth in difficult of exotic habitats, was shown in many parts of the world and the Imperial Forestry Institute of Oxford collected reports from the Forest Services of the British Commonwealth in 1938 (see Rayner 1938).

A.B. Hatch published in 1937 a demonstration by inoculation in pure culture that mycorrhizal Pinus strobus grew faster and absorbed greater amounts of nutrients than infected controls (Table 1). In USA over the suceeding years many experimental studies of the growth of Pinus in nursery beds, with or without mycorrhizal infection were published (Mitchell et al 1937,

McComb 1938, 1943, Finn 1942 etc.).

Table 1. Pure culture inculation of Pinus strobus (Hatch 1937)

Mycorrhiza	Dry weight	Root/shoot	Nitrogen		Phosphorus		Potassium	
			mg	% WT	mg	% WT	mg	% WT
+	304.6	.78	5.00	1.24	.74	.20	3.20	.74
-	320.7	1.14	2.69	.85	.24	.07	1.38	.43

The conclusions were that in soils of moderate nutrient deficiency mycorrhizal seedlings grew faster in height and weight, they tended to have lower root/shoot ratios. By virtue of their increased size they contained more N, P and K per seedling, but in every case they contained more phosphorus, potassium or nitrogen per unit weight and sometimes all three. It was concluded that ectomycorrhizal roots were efficient in absorption of nutrients from the soil i.e. especially that lowest in availability. Mitchell et al (1937) showed that the application of adequate nutrients not only diminished mycorrhizal infection but increased the growth rate; a demonstration that stimulus to growth of the host by infection operated through nutrient absorption. But some experimental results seemed to indicate that mycorrhizal infection increased healthy growth when fertilisation did not, and some eg Lindquist (1939) and Rayner (1944) suggested that mycorrhizal infection exerted a growth-promoting stimulus apart from its effect upon nutrition. The experiments of Rayner and her co-workers with composts upon the infertile soils were very complex. The composts, were themselves complex but said to have little manurial value. They were applied in quantity; the soil was not only infertile but locally toxic. One concludes that there was phosphate deficiency at least, which was offset by compost addition; moreover the C/N ratio and the moisture holding capacity were increased by the compost. The suggested growth promoting effect of the fungus apart from nutrient absorption could not be accepted.

The method by which the fungi increased nutrient uptake was a source of discussion Some thought that the fungi in the soil released nutrients from insoluble sources and they were then absorbed by the host (Burges 1939). This view is puzzling since since the roots are covered by a fungal sheath. However the release of soluble material by fungal activity is a possibility and was examined experimentally by Mitchell et al (1937) and Stone (1950). They failed to find an increase of soluble material, especially N, P and K in the root region of mycorrhizal plants either by analysis or by observation of the growth of associated plants.

One result of Stone's work foreshadowed future advances. He showed that mycorrhizas on Pinus radiata which have copious external hyphae are more efficient in absorbing phosphate from soil than those with smooth surfaces and that mycorrhizal pines diminish the growth and nutrient absorption of competing plants (sudan grass).

At the end of the period under review new techniques became available for physiological experimentation. Kramer and Wilbur (1949), Harley and McCready (1950), Melin and Nilsson (1950) introduced isotopic tracer techniques in the study of nutrient uptake. These and other new techniques allowed many advances, but they had one important defect. Work began on phosphate for the good reasons that it was commonly deficient in soil and that phosphorus isotopes were very convenient to use so workers developed a fixation on the physiology of phosphate absorption.

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Elias Melin: The man and his work

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Elias Melin was born in 1889 and died in 1979, four months before his 90th birthday.

Originality and initiative were not unprecedented among his ancestors. His father Samuel Melin (1849-1931) was a pastor in the Swedish lutheran church, Samuel Melin's father in turn, Anders Svensson (1804-1866) was a farmer. Melin's grandfather (on his mother's side), Carl Wictor Stenborg (1823-1878), was a very active and successful man. At the age of 15, he went to Germany to learn the profession of saddler, and after two years he returned to Sweden and started his own saddler's workshop in Stockholm. Ten years later he married the daughter of his German craftsmaster. When he was 42, his economy allowed him to purchase a beautiful estate (Skämningsfors) in the province of Västergötland, near lake Vättern. He died before his grandson Elias was born, but Elias visited his grandmother and enjoyed the beautiful manor house and garden as a young boy. It is worth mentioning that the grandfather of Carl Wictor Stenborg was an admiral in the Swedish fleet, by name Georg de Frese (1751-1807).

Samuel Melin and his wife Hilda had seven sons and two daughters. Elias was number three in this large family. His six brothers all became pastors in the Swedish church. Only Elias chose a non-theological career.

Elias Melin went to school at the old town of Skara, an ancient bishop's see. In 1907 he started his academic studies at the university of Uppsala. Very soon he joined the large group of students, attracted by the famous professor of plant biology and sociology Rutger Sernander. Melin turned his interest very early towards the genus Sphagnum, and his first publications dealt with the biology and cytology of Swedish Sphagnum species. Hence, he was well prepared when Sernander proposed that he should investigate the problem of establishing to what extent different types of North Scandinavian mires could be used for forest production after trenching.

As a first step, Melin (1917) performed a comprehensive study of the plant associations in different mires. Secondly, he studied the vegetation and, especially, the growth of Scots pine and Norway spruce on trenched peat bogs. In this connection he made the important observation that healthy and vigorous seedlings always had typical ectomycorrhizae, whereas weak and slow-growing seedlings only had "pseudomycorrhizae" (short roots attacked by parasitic mycelia, which penetrate the cells but form neither Hartig net nor sheath). He concluded that, at least on drained peat bogs, the formation of ectomycorrhizae was necessary for normal development and growth of coniferous seedlings. After this discovery he devoted his life to the study of the structure, function and importance of the ectomycorrhiza.

Melin's thesis was given the highest possible grade, "Laudatur", which at Uppsala university has always been used with great restriction. He became "docent" (lecturer) in plant biology at professor Sernander's department, but after a year he moved to the Royal College of Forestry in Stockholm as docent(lecturer) in botany, an office which he held for seven years (1920-1926). In this period, Melin accomplished the fundamental work on the structure and fungal components of the mycorrhiza in forest trees which has given him a world-wide reputation.

Already in his thesis, Melin stated that experimental work was necessary for a deeper understanding of the mycorrhiza, including studies on pure cultures of mycorrhizal fungi and tree seedlings, as well as controlled synthesis of mycorrhiza in vitro. To acquire the necessary experience of microbiological techniques he visited the laboratories of two famous microbiologists on the continent, i.e. Martinus Willem Beijerinck in Delft and Hugo Miehe in Berlin. In 1921 he published a first report on his experimental work in Svensk Botanisk Tidskrift. Here he gave a brief account of the isolation of fungal symbionts from pine and spruce mycorrhizae, as well as of the synthesis of mycorrhizae in axenic seedlings of the conifers by inoculation with the isolated fungal strains. Taxonomic identification of the isolates was not possible, since they did not produce fruit bodies in vitro. Consequently they were given the preliminary names Mycelium Radicis silvestris α, β and γ, and Mycelium Radicis Abietis,

respectively. The presence of clamp connections on the mycelia indicated that they represented Hymenomycetes or Gasteromycetes.

It had been stated by Brefeld (1908), Romell (1921) and others that spores of various Boletus-species did not germinate in laboratory media. Therefore Melin used the tissue-culture method (Duggar 1905) to obtain pure cultures of these and other species, which in nature are associated with specific forest trees. Even this method, of course, was limited due to the fact that a great number of presumtive symbionts could not be cultivated on laboratory media. However, a number of isolates were obtained from various Boletus (sensu lato) species and other hymenomycetes like Amanita muscaria, some Cortinarius-species, Lactarius deliciosus, Russula fragilis, and certain Tricholoma-species.

In 1922 Melin published a report in Berichte der Deutschen Botanischen Gesellschaft on the successful synthesis of mycorrhizae in Scots pine with Boletus (Suillus) luteus and in European larch with Boletus elegans (Suillus grevillei).

In 1923 followed a comprehensive monograph on his experimental research on the structure and ecology of the mycorrhizae in Scots pine and Norway spruce. In the same year he described the ectendomycorrhizae of birch (Betula verrucosa) and aspen (Populus tremula), as well as the experimental syntheses of such mycorrhizae in birch and aspen seedlings, inoculated with species of the genera Boletus (Leccinum), Tricholoma and Amanita.

The methods used by Melin in his experimental syntheses of ectomycorrhizae in conifers were, in principle, simple and effective. During his first experimental period, the working conditions were, however, rather primitive, and his permanent problem was how to avoid contaminations from the air. To solve this problem, he used double flasks, consisting of two combined compartments. The seedlings and fungi were cultivated in sterile sand in the one compartment, whereas nutrient solution could be added to the cultures successively from the second one. In this way, plants could be kept growing for several seasons without contaminations.

During the course of his inoculation experiments, Melin observed that mycelia of mycorrhizal fungi grew much faster in immediate contact with the roots of seedlings than at some distance from the plants. Referring to the works of the Norwegian botanist Hansteen-Cranner (1922) he concluded that organic substances, "phosphatides", were exuded from the plant roots and that certain components of these exudates were necessary for optimal growth of the fungi. The same effect could be obtained by immersing surface sterilized seeds of pine or spruce into synthetic nutrient solutions which were subsequently used for growth experiments with mycorrhizal fungi. By means of this technique, Melin studied the capability of certain mycorrhizal fungi to utilize different nitrogen and carbon sources.

To-day we know, of course, that one component of the exudates was thiamine. However, Melin made his observations long before this vitamin was isolated and identified. Indeed, he concluded that the "phosphatides" affected the mycorrhizal fungi in much the same way as the vitamines of the B-type affect yeast.

The results obtained in the extremely productive period 1917-1925 were summarized and critically discussed in his monograph "Untersuchungen über die Bedeutung der Baummykorrhiza" which was printed by G. Fischer in Jena 1925. In this monograph, Melin presents views on the function and importance of ectomycorrhiza which even to-day give an impression of modern ecological thinking. He emphasizes the competition for available nitrogen between soil microorganisms and plant roots, and he suggests that, in soils of the moor type, the ectomycorrhiza primarily facilitates the nitrogen uptake. However, he also stresses that the ectomycorrhizae of conifers are probably more efficient as nutrient absorbing organs in general than are roots without mycorrhizae, and hence may also be of positive importance in the uptake of other necessary nutrients. The problem of establishing to what extent the mycorrhizal fungi are able to decompose and utilize the more complicated organic components of forest humus is especially stressed.

In the 1920'ies, the possibilities for qualified Swedish scientists to pursue professional scientific work after they had achieved the doctor

degree and thereafter been engaged as docents at the university for seven years were very limited. They had to find a professor's chair. If not, they normally had to accept an appointment as a lecturer at a high school (gymnasium) where there were very limited possibilities for scientific research.

After the period at the Royal College of Forestry, Melin first spent a year with Dr Selman Waksman at New Brunswick, N.J., USA with a grant from the Rockefeller Education Board. During his research on North Scandinavian mires he had become interested in the chemistry and biology of humus and in the laboratory of Waksman he studied the microbial decomposition of different types of litter from North American forests, applying the analytical methods which had been developed by Waksman and his co-workers.

After his return to Sweden in 1928, Melin had to accept a position as a lecturer of biology at a high school in Stockholm. However, as early as in the beginning of the same year, he got the opportunity to apply for a professor's chair in botany at Uppsala - in fact the chair which once was held by Carl von Linnê.

Swedish university history offers many examples of bitter fights between highly qualified scientists for a particular professor's chair. In the case of Melin and his Uppsala chair, the competition and the ensuing discussions were indeed very dramatic. Altogether, the evaluations by the referees, the discussions, and the final verdicts comprised 417 printed pages, and the whole procedure lasted for two and a half years. Melin's main competitor was professor Carl Skottsberg, well known for his excellent work in plant geography and taxonomy. The final appointment of Melin as professor of botany at the university of Uppsala was the beginning of a new era in botany at this university: the era of experimental physiology and mycology.

The sectors of botany which were represented at the university of Uppsala when Melin entered office in 1930 were plant and, especially, algal taxonomy, embryology, plant sociology and plant geography. The need for development into a more physiological direction was strongly felt. From the beginning, as professor, Melin concentrated on two aims, namely (1) the establishment of experimental plant physiology and mycology as an active and respected branch of botany at the university, and (2) the creation of a modern institute with the necessary facilities for teaching and research in this field.

Thanks to his good reputation, pleasant personality, enthusiasm, and skill he very soon attracted a group of students, some of whom became the core of the so called "Melin school". The second aim, i.e. the building of a new institute for plant physiology, turned out to be a more difficult and, indeed, frustrating task. The Swedish economy was in a depressed state in the early 1930-ies. Laboratory facilities which could be used for microbiological research were not available at the old botanical department which had been finished in 1807. So, as a first step, Melin had to take lodgings with more modern neighbouring institutes, first the laboratory of professor The Svedberg and, later on, that of the bacteriologist professor John Reenstierna. In the meantime, however, Melin worked very hard planning a new institute building and trying to persuade the responsible authorities to provide the necessary funds. Stressing the importance of research in basic plant physiology to agriculture and forestry, he was finally able to convince the government that a new department of plant physiology should be built in the botanical garden of the university. Technical details were carefully considered in co-operation with Dr Yngve Björnståhl who had earlier worked with professor The Svedberg in planning and building a new department of physical chemistry. An important principle in these projects was that laboratories should be easily adaptable to the changing demands of scientists. The construction and equipping of the building lasted for almost two years. The building was ready for use by August 1939.

The new department indeed proved to be excellent for experimental work, and an active period of research under the leadership of Melin could start, but it was to some extent hampered by the effects of the 2nd world war. Sweden remained neutral, but all young men had to do military service for longer or shorter periods.

As early as during the period 1934-1939, when Melin was working with a small group of students in rented localities, important results had been achieved. Nils Fries started his career studying the growth promoting effect of certain "lignicolous" bacteria on different wood-rotting Polyporus-species. During a visit to the laboratory of Fritz Kögl in Utrecht, he was able to show that this effect could be substituted for by thiamine + biotin. Working with Kögl he also found that biotin and meso-inositol were necessary growth factors for Nematospora gossypii. Hence, Fries proved that several vitamin heterotrophic fungi could be cultivated in strictly defined synthetic media and in this way opened the field of research on the quantitative needs of fungi for different nutrients, vitamins, and trace elements. The use of defined nutrient media became an important tool in the research activities in Melin's laboratory.

The first student in Uppsla who started mycorrhizal research under Melin was Daniel Lihnell. His interest was the endomycorrhiza of Juniperus communis. This mycorrhiza was treated in considerable detail, but every effort to isolate the fungal symbiont failed. In a subsequent paper, Lihnell described the isolation of Cenococcum graniforme and successful mycorrhiza syntheses with this fungus and Scots pine, Norway spruce, birch, aspen (Populus tremula) and Tilia cordata.

Oskar Modess also joined Melin's group in the early 1930'ies He successfully isolated tissue cultures of several presumptive mycorrhizal species and, using the experimental techniques of Melin, was able to prove that a number of species within the genera Amanita, Boletus (Suillus, Xerocomus), Entoloma, Lactarius, Paxillus and Tricholoma produced ectomycorrhiza in Pinus sylvestris, P. montana, and Picea Abies.

There is no doubt that the most important contribution in the field of ectomycorrhiza, beside Melin's own work, was accomplished by Erik Björkman who in his doctoral dissertation (1942) presented his carbohydrate theory on mycorrhiza formation. Under controlled conditions he found a positive correlation between carbohydrate concentration in the root systems and frequency of mycorrhizae in pine and spruce. Increased illumination, combined with moderate deficiencies in either nitrogen or phosphorus , resulted in increased carbohydrate concentrations in the roots of Scots pine and increased mycorrhizal infection. Björkman's theory, although subject to recent criticism, inspired much research in the following years on the mechanism of mycorrhiza formation. The occupation of the free Baltic States by Soviet troops during the second world war resulted in a stream of refugees, especially intellectuals, from these countries to the West. Several academics found their way to Scandinavian universities. At Melin's department Visvaldis Slankis from Riga in Latvia and some other biologists were offered laboratory facilities. Slankis studied the effect of mycorrhizal fungi as well as their metabolic products on the development and morphology of excised pine roots, grown as aseptic tissue cultures in synthetic media. He found that nutrient solutions from cultures of Suillus luteus and S. variegatus caused swelling and dichotomous branching of short roots, similar to mycorrhizae. Indol acetic acid and related compounds had the same effect. A long and controversial discussion on the role of hormones in the development of ectomycorrhizae began and is still going on.

Appreciating the physiological specialization of the mycorrhizal fungi, Melin was also interested in the problem concerning the deciding physiological differences between these fungi and the saprophytic litter-decomposing basidiomycetes. Such questions initiated the physiological studies by Lindeberg (1944) on the genus Marasmius and by Norkrans (1950) on the genus Tricholoma. In cooperation with Lindeberg and Norkrans, Melin also took up a new line of research which involved the ecological question of how the growth of mycorrhizal and litter-decomposing basidiomycetes is affected by water soluble constituents of different forest litters (Lindeberg 1944, Melin 1946, Norkrans 1950). The main results of these investigations may be summarized as follows:

An addition of small amounts of cold water extracts from various forest leaf litters caused a significant increase in the growth rate of both mycorrhizal and saprophytic fungi, cultivated in a synthetic nutrient medium. Higher concentrations of the same extracts, however, caused an inhibi-

tion of the growth of mycorrhizal species, whereas the stimulating effect on the litter-decomposing species increased with increasing concentrations. The stimulating effect was partly due to inorganic ions, mainly calcium and manganese (Lindeberg 1944), and partly to unidentified organic factors.

In the early 1940'ies, the identification of the active organic components would have required very extensive and complicated analytical procedures, and the question of their chemical nature was left open. Much later, Olsen, Odham and Lindeberg (1971), using gas chromatography and mass spectrometry, were able to show that the inhibitory effect of aspen litter extracts on mycorrhizal species was mainly due to benzoic acid and catechol.

Melin quickly appreciated the possibilities of isotope tracer techniques in mycorrhizal research. In co-operation with Harald Nilsson he used these techniques to prove the fact of the transfer of products of photosynthesis from pine seedlings to the fungal symbiont, as well as of the transfer of nitrogen, phosphorus and calcium from the substrate via the fungus to the plant. (Melin & Nilsson 1950-1958)

In later years, Melin's interest was mainly focussed on those root exudates which stimulate the growth of mycorrhizal fungi, i.e. the so called "M-factor". The effect of this factor was demonstrated in experiments where excised pine roots, cultivated as tissue cultures, were placed on the surface of nutrient agar, containing suspensions of finely divided mycelia of different mycorrhizal fungi. The stimulating effect of the root exudate on the growth of the fungi was very evident. Several attempts were made to purify and characterize the active principle but, much to Melin's disappointment, no definite results were obtained.

In his scientific career, Melin showed a logic development from keen observations of nature to critical physiological laboratory experiments, finally approaching the biochemical aspects of mycorrhizal research. He had an outstanding ability to formulate the problems, to attack these problems by adequate and, if necessary, new methods, and to present his results logically and critically. If inferior work was presented in his field, his criticism could be merciless. His self-confidence was strong and his ability to encourage his students was exceptional.

Several visiting scientists from abroad worked for shorter or longer periods with Melin - Hatch, Mikola and Hacskaylo to mention but a few. They enjoyed the inspiration of Melin's enthusiasm and leadership and contributed themselves to the development of the Uppsala mycorrhizal research centre.

Melin involved himself in the work of his students very closely, generously sharing ideas and viewpoints in discussions which, very often, took place late into the night, long after the administrative work had finished. The sacrifices this meant to his family were certainly considerable. His wife, Margit Melin, however, showed unfailing loyalty to her husband and his work. Together they played a highly valued role in the social life of the botanists in Uppsala.

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ADVANCES AND QUIESCENCE - PATTERNS OF MODERN RESEARCH ON ECTOMYCORRHIZAE

By

Edward Hacskaylo

Introduction

In 1949, Dr. W. W. Diehl at the George Washington University, suggested that I consider mycorrhizae as a potentially rewarding pursuit for graduate study. I soon realized that there was little interest in mycorrhizae in the United States and no institution seemed prepared to provide guidance to a graduate student wanting to work in that area. However, continuity in ectomycorrhizal research had persisted in neutral Sweden through World War II. At the University of Uppsala, Elias Melin and several of his students established, and subsequently maintained, a position of strong research leadership. Consequently, I sailed for Sweden in the autumn of 1952 and spent the following fifteen months in Professor Melin's Institute in Uppsala on a pre-doctoral fellowship howping to acquire data for a doctoral dissertation. I also wanted to gain the best possible insight into the subject of mycorrhizae.

The number of mycorrhiza researchers in the world in the 1950's was very small. Most were in contact with each other through correspondence, literature exchanges, personal visits and meetings. Research progress was slow and it was relatively easy to keep abreast of "all there was to know about the subject." By the late 1960's the research tempo had quickened. Increased communication between researchers at international meetings and dissemination of information about mycorrhizae through publications and lectures seemed to crystallize the validity and significance of mycorrhizal relationships. Through the 1970's to the present, the number of mycorrhizasts has become very large and there is general acceptance of the importance of mycorrhizae. Most plant scientists have at least some knowledge of the subject.

There have been fascinating research transitions in ectomycorrhizal research during the past forty years. However, rapid advances in specific areas have usually been followed by long periods of little progress. Continuity in lines of ectomycorrhizal research has not been the pattern in most laboratories.

I should like briefly to comment on certain events and personages and their influence on the development of the general field of ectomycorrhizae since World War II. I shall not include specific literature citations since they are easily attainable elsewhere. The failure to mention any particular mycorrhizast in no way is intended to diminish the importance of his or her contribution.

Uppsala and the elusive M-factor

The thrust of ectomycorrhizal research was in physiology and ecology as we emerged from World War II. In Uppsala, Elias Melin led students Erik Björkman, Birgitta Norkrans, Nils Fries, Daniel Lihnell, Oskar modess, Gösta Lindeberg, and Visvaldis Slankis through doctoral studies performing precisely planned and executed experiments. Each exposed vital facets of ectomycorrhizal physiological relationships, sometimes controversial and often still unresolved.

Not long after I had started to study about mycorrhizae, I met Melin's student Birgitta Norkrans who was visiting the Mycology Laboratory at Beltsville. I commented to her that the only way it appeared that I was going to really become educated about mycorrhizae was to go to Sweden to study. She was very encouraging about the possibility and, as I mentioned previously, it became a reality a couple of years later.

When I arrived in Sweden in 1952, Norkrans and Nils Fries were still doing research and some teaching at the Institute of Physiologial Botany. However, they were then working with non-mycorrhizal fungi. I found them communicative and helpful to the students and other researchers at the Institute. Perhaps they followed the example of Melin who had a little electric sign at his office door nearby was the doorbell button which one could always push. If he were busy the sign would light up indicating that, or if he were not, another little sign would light up saying "Stig På" ("come in"). He always seemed to find time to talk with his students and co-workers in a helpful and friendly manner that made us feel very comfortable. Melin was very fond of the students and the university life at Uppsala. He was the "Inspektor" (advisor) of the "Nation" (student organization) that represented his native province Västgötland. Every student at Uppsala belonged to one of the provincial "Nations", including those of us from abroad. I joined Melin's "Nation" and participated in many social festivities. Melin was also a great participant in activities amongst the students at the "Nation" and often at his home. Mrs. Melin, a forthright, outgoing and gentle lady, likewise was very close to the lives of the students at the University.

Björkman, Modess, Lihnell, Lindeberg and Slankis had graduated from Uppsala and were in positions elsewhere before I arrived there. I did not meet them then, but know their work very well.

In the mid 1940's Melin began studying root metabolites that appeared to affect the relationship between host and fungus. One of the major things he found was marked stimulation of mycorrhizal fungi when grown in the presence of excised living roots in axenic culture. This became the elusive metabolite Melin called M-factor. Using the then new technique of paper chromatography, and others such as diffusion through membranes, he attempted to characterize the nature of the M-factor. When I was in his laboratory I witnessed many of his extensive experiments and also had the opportunity to do some experimentation M-factor caused a very real stimulus to mycelial growth and to

germination of basidiospores of ectomycorrhizal fungi. However all attempts to chemically identify that factor have failed. Melin continued to experiment with the M-factor well up into his eighties in collaboration with other scientists in Sweden.

The last conversation I had with Melin about the M-factor was at his summer home in central Sweden in 1976. I asked him if he had any additional information on the M-factor and he replied that the conlusion he came to, after looking at data of cooperating biochemists, was that it probably was not a single factor but a complex of factors. Recently Nils Fries and coworkers have used the M-factor principal for germination of basidiospores of ectomycorrhizal fungi in vitro including Thelephora terrestris, a fungus which I had also worked with.

To me, M-factor is one of the most intriguing questions in ectomycorrhizal associations. It should be studied using techniques that were not available in 1950 to bring the speculation as to what it might be to a conclusion. There are those who steadfastly insist that the M-factor is an aberration in experimentation. I, personally, do not agree. If someone can produce better reasons for those conclusions that I have heard until now, I would accept a review of the subject and perhaps change my impressions about what I have seen. This neglected area has vital potential and has been dormant for too long.

Growth substances - "on a postage stamp?"

In the years immediately after World War II, studies on plant growth substances flourished. It seemed logical that infections of roots by ectomycorrhizal fungi would become suspect as centers of some type of hormonal action. In meticulously executed studies, V. Slankis found that exudates from certain ectomycorrhizal fungi grown in liquid culture as well as synthetic indoleacetic acid and napthaleneacetic acid could induce roots of Pinus sylvestris to branch dichotomously and superficially appear very similar to ectomycorrhizae. He did not study the internal cell morphology of the treated roots. Slankis developed the idea that fungus auxin was a very essential part of the mycorrhizal process and for several years proposed extensive theoretical implications of auxin in establishment and maintenance of the symbiotic association. Limited studies by M. Moser, J. Ulrich and others demonstrated the potential for auxin production by ectomycorrhizal fungi in the presence of tryptophane. Many questions have been raised about the role of fungus auxin in ectomycorrhizae.

In 1967 André Fortin found evidence that auxins produced by external organisms or from an external source could be inactivated by oxidases in root tissue. His results did not indicate that fungus auxin was necessarily directly responsible for morphogenic manifestations in ectomycorrhizae. Others suggest that the infection by ectomycorrhizal fungi interferes with inactivation of auxin produced within root tissues and consequently internal accumulation is responsible for the changes in the morphology and attending physiological effects caused by increased auxin concentrations. I've found that the presence of auxin in fungal cultures is very difficult, often impossible, to detect with soybean callus assays. Although there are morphological and physiological characteristics of ectomycorrhizae that might be influenced by fungus auxin, it seems that the question of auxin activity is in need of much more intensive study.

While discussing plant hormones it is appropriate to mention the work of Carlos Miller who undertook investigations on the potential role of cytokinins in mycorrhizal associations. One of the first fungi he tested, Rhizopogon roseolus, produced zeatin riboside abundantly. He and C. Krafts surveyed several groups of fungi and found the production of cytokinin to be very erratic among the ectomycorrhizal fungi, not only in quantity produced, but also in the ability, or lack of it, of different species to produce cytokinins. We found the same using Miller's callus tissue assay techniques. I have heard it said that what we know about plant hormones in mycorrhizal relationships could be "put on the back of a postage stamp."

There could seemingly be many benefits of fungus-produced auxin and cytokinins in etomycorrhizal associations but thus far such compounds do not appear to be products of all ectomycorrhizal fungi. We therefore question their universal occurrence in this symbiotic association. Although of potential importance, the involvement of hormones in ectomycorrhizae have not been systematically studied. Hopefully, they will be explored further and their role in mycorrhizal associations, if any, will be clarified.

Carbohydrate controversies

Erik Björkman's dissertation in 1942 on physiology and ecology of mycorrhizae of Pinus sylvestris was widely distributed in forestry circles. Björkman was an enthusiastic individual who approached the subject of mycorrhizae very seriously and with great vigor. Among his many contributions to the literature on mycorrhizae, the attempt to define the mechanism controlling ectomycorrhiza formation was probably most significant and controversial. He hypothesized that since mycorrhizal fungi can assimilate only soluble carbohydrates the absence or presence of sugars within the roots can be directly correlated with formation of mycorrhizae; mycorrhizal fungi will not penetrate the roots unless the roots contain a certain quantity of "surplus soluble carbohydrates." Björkman used tissue analyses for reducing substances to determine the quantities of soluble carbohydrates in the roots.

Initially the hypothesis was favorably received and was not experimentally challenged for 20 years. W.R.C. Handley and C.J. Sanders, in 1962, attempted to duplicate Björkman's studies and concluded that the increased amount of reducing substances was a result of accumulation

of carbohydrates in the fungal mycelia. F.H. Meyer, also in 1962, working with Fagus sylvatica seedlings, thought that the increased sugar content was induced by mycorrhizal fungiand other microorganisms in the soil. He later also suggested that auxins produced by the fungi promote hydrolysis of carbohydrates in the tissues and prevent the accumulation of starch grains in the stele; supposedly the fungi could then absorb carbohydrates from the tissues and increase the flow of suluble carbohydrates from stems to roots. W. Schweers and Meyer performed further experiments, the results of which they interpreted as support for the concept that rising levels of sugars in the root are the result of root infection by mycorrhizal fungi and not the cause. Slankis, in 1971, expressed the opinion that soluble carbohydrates are not the sole factor in formation of ectomycorrhizae. He found that when nitrogen and phosphorus concentrations in the nutrients are increased mycorrhizal roots return to a nonmycorrhizal state. His results were similar to those of earlier investigators. However, he did not find that a decrease or deficiency in soluble sugars in the roots reversed the mycorrhizal process and then he too took issue with Björkman. Slankis proposed a major role for fungus auxin in establishing and maintaining the symbiotic relationship. He conceded, however, that there seemed to be a correlation between light intensity and mycorrhiza frequency but that the action of light was not clear.

Harley also questioned the validity of determining carbohydrate content by analyzing for reducing substances because easily soluble reducing substances normally contain compounds other than sugars, and because preparatory treatments might have resulted in formation of new and different reducing substances. He didn't reject the premise that soluble carbohydrates could promote mycorrhizal formation but suggested that copper reduction techniques used by Björkman were not specific for carbohydrates. Björkman very vigorously defended his hypothesis and in 1970 again presented data to support his ideas. He was unable to find increases in the sugar content of roots as a result of introduction of a mycorrhizal fungus and criticized experiments of others which were performed during times of the year different from the times he conducted his. Unfortunately the debate was terminated with the untimely death of Björkman in 1973.

I have performed several experiments on nutrition, light intensity, and photoperiod as they affect ectomycorrhial formation. My data tend to support Björkman's conclusions. High levels of nitrogen and phosphorus, low light intensities, dormancy of seedlings induced by short photoperiods, blocking of photosynthesis by covering the leafy area with a barrier to light, all tend to reduce production of photosynthate and diminish or eliminate ectomycorrhizal formation on Pinus virginiana.

The fine work of D. H. Lewis and Harley in the 1960's on translocation of carbon compounds from host into associated mycelium extended our knowledge on the fate of carbon compounds in ectomycorrhizal associations. Their work signified an important transition from general physiological and ecological experiments performed by the Swedish school to a biochemical, analytical approach characteristic of the experiments performed by Harley and his students.

Lewis and Harley analyzed mycorrhizal and non-mycorrhizal roots of beech, detected trehalose and manitol in mycorrhizae but not in uninfected roots. They found that conversion of glucose and fructose to trehalose and manitol, plus synthesis of glycogen in the fungus established a concentration gradient; hence transport of carbohydrate occurred in the direction of the fungus. Lewis and Harley concluded that translocation of sugars into the fungal sheath tends to conserve a supply of carabohydrates for the metabolic processes of the associated fungi. The sheath acts as a physiological sink and restricts movement of sugars back into the host. Melin and Nilsson had traced C^{14} applied as CO_2 to needles of P. silvestris down through the shoots and roots into mycelium of the attached mycorrhizal fungus Boletus (Suillus) variegatus. However, C. P. P. Reid and F. W. Woods found movement of C^{14} reciprocally between Thelephora terrestris mycelium and roots of Pinus taeda through connecting mycelial strands. These and other experiments demonstrated clearly that mycorrhizal associations and indeed survival and reproduction of ectomycorrhizal fungi is directly linked to the photosynthetic activity of the host. The respiratory consumption of carbohydrates by associated fungi has been estimated by Rommell and by Harley at 20-25% of total root CO_2, and the sporophore production equivalent to 10% of the potential timber stand. Although these are representative figures, they are no doubt good as indicators of the magnitude of the carbon role in ectomycorrhizae.

In a different approach to carbohydrate metabolism, Norkrans, in the 1940's, worked on the production and action of cellulolytic enzymes of species of Tricholoma . Her work demonstated that certain species possessed the capability of decomposing cellulose but those species were not the most obligate as mycorrhizal associates. Occasionally since then reports have emerged from other laboratories on the potential for cellulose degradation by mycorrhizal fungi. The extent and importance in ectomycorrhizal associations seems to be quite limited but there is the possibility of saprophytic existence as well as obligate symbiosis by certain ectomycorrhizal fungi. Bruchet and I studied the saprophytic yet mycorrhizal nature of certain species within that genus while others had obligate symbiotic characteristics. The potential for saprophytism or facultative relationships may be more prevalent than we previously believed.

These few highlights are indicative of the status of our still limited knowledge in carbon metabolism in ectomycorrhizae. Much has been revealed in fragments but conjecture remains on the composition of carbon compounds as they move

across the fungus and host membranes.

Interactions of mineral nutrients and carbon compounds is a complex subject that can only be mentioned here. R. C. France and C. P. P. Reid reviewed nitrogen and carbon interactions in ectomycorrhizae at the last NACOM but it needs to be emphasized that a vast area of research remains as a formidable challenge.

Technology accelerated nutrient uptake studies

In the early post-war years a brief article occasionally would appear in the United States on mycorrhizae that would be of significance. P. J. Kramer and K. M. Wilbur, in 1949, reported on greater absorption of radioactiave phosphorus by mycorrhizal than non-mycorrhizal roots of pine. This was the first published report on use of radio isotopes as tracers in movement of minerals into mycorrhizal plants. At the same time, Melin and H. Nilsson were experimenting with uptake of radioactiave phosphorus and its movement into pine seedlings by mycorrhizal hyphae. In 1950, they published the first of a series of experiments on the transport of labelled phosphorus, nitrogen, calcium, and cations in the form of sodium, into seedlings of pine from the substrate through mycorrhizal mycelium. Those classic experiments proved conclusively that ectomycorrhizae translocated nutrients into host plants more effeciently than nonmycorrhizal root systems. Meanwhile, Harley and his group at Oxford were working on uptake of phosphate by excised mycorrhizal roots of beech. The first of their papers also appeared in 1950. Many additional papers from Harley's laboratory followed in the ensuing years. Those experiments were not limited only to phosphate uptake but encompassed studies on accumulation in the sheath, on movement into the host tissues, on the enzyme systems and, indeed were usually indepth biochemical investigations.

Harley and his co-workers provided basic insights into absorbtion of other inorganic and organic compounds including potassium, sodium, and rubidium. We were particularly impressed with their findings that nutrients could be stored in the sheath in a pool and then released into the plant when an external source was no longer available and by their technique of blocking enzyme systems to study accumulation and transport of ions.

In the early 1960's, Glynn Bowen, in Australia, came onto the scene in mycorrhiza literature. Bowen, initially interested in the fate of the phosphate within the plant expanded into mechanisms involved in absorbtion by mycelial strands in the soil and movement through those strands into the host plant. He has consistently pursued the objective of synthesizing data on mycorrhizae. He stated, "Mycorrhizae influence nutrient uptake, carbohydrate distribution, and growth substance production, and our eventual aim should be to understand how these factors are integrated in plant growth in soil."

Nutrient uptake is too vast to discuss in detail here. However, I should like to suggest that it is not sufficient to focus on identifying the elements that are absorbed from the soil - try to measure the quantities. An ectomycorrhiza is not limited to the small lateral root with its mantle and Hartig net. Hyphal strands extend into the surrounding soil and are dynamic segments of the ecosystem that are scarcely recognized. What is the role of ectomycorrhizae in nutrient recycling? What portion of the annual turnover of plant tissue do they represent? Extensions of these studies reported recently by K. Vogt and her co-workers and by R. Fogel accentuate the appropriateness of Bowen's comment. Nutrient uptake has moved toward a new dimension that we will watch with great interest.

Taxonomists are catching up

Distinguishing one mycorrhiza from another on the basis of the associated fungus is a formidable task. Various approaches have been used for fungal identification and verification of their mycorrhizal habit that range from observational data on associated sporocarps, to tracing mycelia between roots and sporocarps, to using clearly disktinguishing features of mycorrhizal fungus mycelia and finally to axenic culture synthesis of the host and fungus. The latter is considered to be the most reliable. Although taxonomic descriptions of mycorrhizal fungi often refer to associations with higher plant species, recognition of their mycorrhizal status has developed slowly.

In the 1950's, R. Singer specifically attempted to establish fungus-host mycorrhizal relationships as a feature of interdependency in distribution of both symbionts. Later, J. M. Trappe identified many species of fungi as suspected mycorrhizal partners in the Pacific Northwest that culminated in publication of all fungus/host associates he could find in the literature as of 1962. Trappe developed a special interest in hypogeous mycorrhizal fungi that encompassed both ecto- as well as endomycorrhizal species. The ecological studies of Trappe and his co-workers on ingestion and distribution of mycorrhizal fungi by small animals relates very positively to colonization and distribution of the hosts and their associated fungi.

We emphasized taxonomy as a basic, important tool in mycorrhizal research at the first NACOM when A. H. Smith presented a discussion on taxonomy of the Basidiomycetes, and J. W. Gerdemann on the developing taxonomy of the mycorrhizal Endogonaceae. It is significant and gratifying that taxonomy of ectomycorrhizal fungi is included in recently published books on identification of mushrooms like O. K. Miller's "Mushrooms of North America."

Identification of ectomycorrhizal fungi from mycelial cultures was intensively researched by R. Pachlewski and his wife during the 1960's. They extablished criteria for identifying many European ectomycorrhizal fungi but there has been little application of their studies. Recently, O. K. Miller, J. G. Palmer and their students reported progress in establishing a key for identifying mycelial cultures of certain

ectomycorrhizal fungi based on morphological and biochemical characteristics. They correctly assume that mycelia of species and isolates of ectomycorrhizal fungi will be different. However, whether they are sufficiently different to permit identification on a wide scale remains unknown.

On classification and terms

Periodically through the history of mycorrhizal research there surfaces an apparent feeling of need to systematize ectomycorrhizae on the basis of physical characteristics. Melin had constructed a simple classification of ectomycorrhizae in 1923. Hatch and K. D. Doak wanted to slightly modify his system by eliminating the term "pseudomycorrhiza." Many years later T. Dominik proposed an elaborate classification of ectomycorrhizae which some of us attempted to use but found it to be burdened with complexity and ambiguity. The system also had no mechanism to compensate for morphological changes within a single mycorrhiza as it aged and senesced. There were few combinations of host and fungus that were consistently identifiable except in regions with small numbers of species of symbionts. However, Dominik's system has been used successfully on a limited scale for comparisons of ectomycorrhizae, particularly in central Europe. B. Zak in 1969 resurrected the potential need for a classification system for ectomycorrhizae but there has been little progress toward practicality.

While on the general subject of classification I should like to include some comments on terminology. The most outstanding modification in our mycorrhiza vocabulary was proposed by B. Peyronel et al. in 1969, who suggested eliminating "trophic" from mycorrhizal classifications. They streamlined the terms to relevancy and easy usage. I'm not quite so relaxed with the designation of mycorrhiza research proposed at the 3rd NACOM in Athens, Georgia. "Mycorrhizast" isn't the most euphonious word I ever heard but perhaps it fits the need. J. L. Harley receives whatever credit is due for its introduction. Will they ever accept -a, -ae endings?

From Cenococcum to E-strains and beyond

Peitsa Mikola received a Finnish fellowship to do research in Melin's laboratory in the late 1940's to study the physiology of Cenococcum graniforme. Mikola retained interest and active involvement in mycorrhizal research up through retirement and into post-retirement years. He had a unique talent for correlating the significance of mycorrhizae with practical silvicultural perspectives. He and his graduate student O. Laiho made the first important inroad into the isolation and identity of ectendomycorrhizal fungi. Mikola and Laiho proved that ectendomycorrhizal fungi of pine nurseries were a group apart from those forming ectomycorrhizae or endomycorrhizae. Followup investigations by others continue and are beginning to make some sense. The very careful work of Hugh Wilcox and his coworkers and more recently of R. M. Danielson appear to be rapidly advancing toward characterizing and identifying many ectendomycorrhizal E-strain fungi. No doubt the importance of these fungi will emerge as greater than has been perceived in the past.

Mikola became Professor of Silviculture at the University of Helsinki where he kept an eye on promoting mycorrhizal research and advancing the potential of mycorrhizae world-wide.

An effort was instigated by Erik Björkman through the International Foundation for Science based in Stockholm, Sweden to support research on mycorrhizae in developing nations by granting funds for training, attendance of meetings and research to noteworthy participants in smaller countries where funding was difficult to obtain. Björkman helped to establish the program. His death did not terminate the activity, since Mikola took his position and chaired the mycorrhizal research effort in IFS. He has served as a consultant to many young researchers in nations around the world and has helped to establish programs of research in those countries. This has abeen a very commendable effort on the part of IFS, Björkman, Mikola, and the others who have cooperated in assisting the scientists from those countries.

Is there practicality to inoculations?

Since the early part of this century, exotic species, particulary of pines, have been introduced into many parts of the world and successful introduction was recognized as dependent upon infestation of the soil with mycorrhizal species of fungi. That usually was accomplished with imported soil containing mycorrhizal fungi from another region or country. In the post World War II era we began to see refinements of the procedures for the effects of pure culture inoculations. Pure culture inoculations were not commonplace although there were reports, for example, by Ida Levihson that pure mycelial cultures were used for successful establishment of mycorrhizae on pine.

A method for producing spawn of mycorrhizal fungi was reported in the late 1950's by M. Moser in Austria. He grew fungi in autoclaved peat moss moistened with a sterilized nutrient solution, inoculated seedlings and recorded differences in stimulation of their growth. There were other reports of successful field inoculations in the Soviet Union by scientists where oak species had been inoculated with spores and mycelia of ectomycorrhizal fungi.

In 1959, I had my first opportunity to evaluate the effects of inoculation of pines with soil that contained ectomycorrhizal fungi in Puerto Rico. Pinus taeda trees were, without question, stimulated by the development of mycorrhizae on their root systems. Those which did not develop mycorrhizae did not survive. I sent additional soil from a pine woods in Maryland to Puerto Rico that was used to inoculate seedlings in the nursery to ensure they were mycorrhizal before being outplanted. The results were very successful. In 1965, J. A. Vozzo and I

inoculated Pinus carribea seedlings with pure cultures and with a mixture of fungi and with soil from existing pine stands. Stimulation of the growth of pine seedlings occurred when mycorrhizae formed either with pure culture or soil inoculation. For some unknown reason the mixture of organism in the soil resulted in greatest stimulation of the seedlings. Whether this was due to the mixture of organisms on or around the root surfaces, or to one particular fungus in the mixture is not known. I should like to have had the opportunity to follow up on

that potentially rewarding pursuit. I did, however, later find several same species of fungi fruiting in Puerto Rico that occurred at the source of the soil in Beltsville. This meant that over a period of 10 years the fungi persisted from the original small amount of inoculum through to maturing pine stands.

The experiments in Puerto Rico received considerable attention internationally, and in 1963 a group under sponsorship of the IUFRO Mycorrhiza Working Party met there for onsite observations on inoculation experiments. Reports were prepared by Erik Björkman and by Peitsa Mikola who were Chairman and Co-Chairman, respectively, of the Working Party.

In order to evaluate the potential of mycorrhizal inoculations, Mikola spent most of 1968 travelling to locations throughout the world where reports existed on the use of inoculum to establish mycorrhizae on forest trees. Meanwhile, with continued growing interest in the potential for inoculations in the United States, the U.S. Forest Service became involved in developing technology to make practical application of mycorrhiza inoculations in forest nurseries under direction of B. Zak. D. H. Marx worked as a laboratory technician with Zak and later progressed through graduate studies into specializing first on production of antibiotics by ectomycorrhizal fungi, and then on the development of inoculation techniques and procedures for practical applications in nurseries. Marx met this challenge with a tremendous amount of enthusiasm. By applying information already known, followed by improved technical methods, he was able to test the efficacy of a particular mycorrhizal fungus, Pisolithus tinctorius, in establishment of pines, particularly, in soil sites that were very hostile environments for establishing plants. Pisolithus tinctorius, as J. R. Schramm in 1966 had observed on anthracite spoil banks, is a very vigorous colonizer on certain adverse sites. It grows very rapidly and fruits abundantly; producing huge masses of spores. Marx capitalized on these features and found that he could successfully grow P. tinctorius in a sterile peat moss and vermiculite medium moistened with a nutrient solution; then wash it with water to remove excessive nutrients and apply the medium that contained mycelium, particularly, within the lamellae of the vermiculite to natural soils. The resulting stimulation of pines was indeed very dramatic. He established extensive test plots throughout North America and other parts of the world in cooperation with many nurserymen and individual scientists. His experiments have shown the potential practicality of establishing mycorrhizae prior to outplanting on the adverse sites. Although many others have duplicated Marx's experiments and there are serious efforts to commercially produce inoculum, it would be premature to express an opinion on the ultimate outcome of widespread attempts to inoculate forest nurseries. Hopefully, the potential remains one with great promise.

Closer and closer scrutiny still needed

The gross morphological features of mycorrhizae are quite distinct from uninfected lateral roots. The early literature often described superficial characteristics of ectomycorrhizae. The morphology of mycorrhizal root systems had been approached by several investigators including Melin, Hatch and Doak. In the post-war period, F. A. L. Clowes made a thorough study of the developing root system and of mycorrhizae of beech that established a basis for studies that would follow. He observed that there was really no hyperplastic response to the invasion by the fungus, but rather reorientation of the cells so they tended to be isodiametric. Among other details, he did not find a particular increase in volume in mycorrhizal cortex cells so one would not conclude that hypertrophy was involved to any great extent. J. G. Palmer, in the early 1950's, did similar studies in measurements with Pinus virginiana and drew similar conclusions.

During this period Noel Robertson found that long roots of Pinus sylvestris, as well as short roots, could become infected by mycorrhizal fungi and the infection of newly formed short roots could arise from the hyphae within the long roots. Hugh Wilcox followed in the late 1960's with very fine reports on studies on morphogenesis of roots and the infection process in Pinus resinosa mycorrhizae. A. von Hofsten in Sweden and S. Scannerini and his associates in Italy carried investigations on morphology of ectomycorrhizae into further detail using electron microscopy.

More recently J. E. Nylund in Sweden while studying the morphogenesis of ectomycorrhizae reported, among others, two salient points which I believe mandate more investigation on the development of ectomycorrhizae. He reported that plasmadesmata can be found connecting cortical cells through the Hartig net in ectomycorrhizae of pine. This should be reexamined closely by others, particularly in light of claims that once the cortex is infected the root cells tend to become moribund. The presence of plasmadesmata should provide a mechanism for survival of and movement of nutrients between cells that are physically separated by the Hartig net. The other point was that until a labyrinthic formation of Hartig net hyphae was differentiated the mantle did not assume the characteristic pseudoparenchymatous structure. Whether that sequence takes place in other than his experimental material certainly requires additional study.

In the ontogeny of ectomycorrhizae we assumed

that the ectomycorrhizal fungus secreted pectolytic enzymes enabling the hyphae to penetrate the intercellular regions of the cortex. However, B. Lindeberg and M. Lindeberg studied the production of pectolytic enzymes by ectomycorrhizal fungi. They were unable to detect pectinase activity in cultures of the fungi but left open the possibility of enzyme production at the hyphal tip in contact with root tissues. Other more recent studies appear to confirm the Lindeberg's negative results. Electron micrographs show at least some entry of hyphae by mechanical separation of the cells. It is my impression, however, that both mechanical and enzymatic separations should be considered in future studies.

Genetic manipulation

K. L. Giles and H. Whitehead have reported the only instances of which I am aware on attempts to incorporate N-fixation capability into ectomycorrhizal fungi. This they reportedly accomplished by fusing the sphaeroplasts of Azotobacter vinelandii with Rhizopogon roseolus. They noted, however, that one strain of the modified fungus tended to become pathogenic toward roots of Pinus radiata. Although there are formidable problems involved, the natural compatability of mycorrhizal fungi with root tissues of higher plants should give priority to modifying mycorrhizal fungi to fix nitrogen over attempts to modify the genetic constituents of root cells. The transfer of nif-genes or incorporation of sphaeroplasts, organelles or DNA from bacteria or Actinomycetes into the true fungi might be more easily attainable than attempting to incorporate the N-fixing capability into root tissues. I see no reason to doubt that the knowledge and techniques will eventually exist to allow alteration of the genetic constituents of mycorrhizal fungi. This might include tolerance to ranges in temperature, soils, pH and moisture levels, increased efficiencies in nutrient uptake including N-fixation, wider ranges of host-fungus compatibility, and even selectivity toward most desirable edible sporocarps. Long-range planning and intensive research efforts will determine the feasibility of genetic manipulation.

Major publications

Other than scientific papers, the first book that attempted to bring the literature on mycorrhizae under one cover was by A. P. Kelley in 1950. The book did not fulfill the needs of synthesizing and interpreting available data on mycorrhizae but the extensive bibliography was particularly valuable for those who were just entering the field. Books written by N. W. Lobanov in 1953 and by N. M. Shemakhanova in 1962 in the Soviet Union were quite inclusive and were good sources of data, particularly of East European and Soviet researchers.

The book which no doubt had the largest impact on mycorrhizasts was "The Biology of Mycorrhiza" by J. L. Harley, published in 1959 followed by a second edition in 1969. Well-written, it synthesized and interpreted data and was an excellent reference source and text book. In 1971 the proceedings of the 1st NACOM appeared, followed by the specialized book, "Ectomycorrhizae," edited by G. C. Marks and T. T. Kozlowski. Many of us contributed chapters to that volume and it, too, was an excellent resource for updating and interpreting information available at the time. The Proceedings of a meeting on tropical mycorrhizal research held in Ghana in 1979, edited by Peitsa Mikola, was oriented toward establishing forests, particularly in developing tropical countries. In 1983, a methods manual for mycorrhizal research appeared. Edited by N. C. Schenck, it contains contributions primarily by American authors and is intended to ease the burdens of those embarking on mycorrhiza studies.

The latest book of interest is "Mycorrhizal Symbiosis," by J. L. Harley and S. E. Smith, father and daughter. I received a copy prior to this meeting and have tried to get an impression of its contents. The first portion of the book updates information on the general aspects of mycorrhizae and the latter portion is devoted to a series of essays on several specific topics. The authors have attempted to bring into perspective the relevance of past research and to indicate possible directions for future research.

They have relied heavily on data from fields other than mycorrhizae for interpreting and evaluating physio-biochemical processes which might occur in both ecto- and endomycorrhizae. The essays are written so they may be read individually and will require careful study before a reasonable evaluation can be given regarding the merit of their conclusions. Portions of the essays will be controversial but there is no doubt that they will stimulate interest and discussion, and, possibly, stimulate future research in certain areas.

There have been a few other compilations on mycorrhizae that have appeared either as parts of books or as small books. However, although they are significant since they have tended to reach broader audiences than mycorrhizasts alone, many of the data in them can be found in the publications that I have mentioned.

The impact of communicating

As I stated earlier, increased communication between researchers has had enormously stimulating effect on research on mycorrhizae. Until 1956 no organization had fostered meetings or communication specifically between mycorrhizae researchers in any part of the world. However, at the International Union of Forestry Research Organizations Congress held at Oxford University in that year, Erik Björkman organized a Working Group that was to become a major forum and coordinating group for mycorrhizasts. Fifteen individuals including some who are present at this meeting, Professor Harley, Dr. Mosse and I, were asked to participate in the Working Group to help foster better communication between mycorrhiza workers.

In 1959, Björkman also organized a session at the International Botanical Congress in Montreal where some of us presented papers. Among them were interesting reports on endomycorrhizal fungi by J. T. Barrett of the University of California, who had isolated an organism he believed was an endomycorrhizal fungus in the Endogonaceae, and a paper by Barbara Mosse on her endomycorrhizal work. S. A. Wilde from the University of Wisconsin presented a paper and left us with the impression that he, at that time, believed that mycorrhizae were important but he was not sure that actual invasion of the root had to take place for the effect to be significant. He also indicated that, perhaps, unless one were working in an area such as the prairie soils where ectomycorrhizal fungi were not indigenous, one should not be terribly concerned about inoculations. Once the fungus was introduced he thought mycorrhizal infection would take place spontaneously. That meeting was indeed a landmark for us because it started to bring together mycorrhiza researchers at international gatherings.

In 1961, the working group met at the Congress in Vienna, Austria, where we presented papers at a session specifically set aside for mycorrhizal research. Björkman served as Chairman of the IUFRO group until his death in 1973. Peitsa Mikola then became Chairman. We continued to meet at the IUFRO Congresses and, in 1976 in Oslo, two interested groups merged -- the Mycorrhiza Working party (so called at that time) and a group interested in tree root physiology under the leadership of Arthur Riedacker of France. At an organizational meeting we established the Root Physiology and Symbiosis Working Party within IUFRO. I became Chairman and Riedacker and R. van den Driessche of Canada were Co-Chairmen.

Riedacker suggested that we meet before the next IUFRO Congress in collaboration with the French root research group at their annual meeting. The first independent meeting of the Working Party was held in Nancy, France, in 1978 that was broader than symbiosis alone and included many other aspects of root physiology and morphology. The Working Party held its second meeting in Edinburgh and Canterbury in 1982 and plans to meet approximately every 4 years.

At the AIBS Meetings at the University of Illinois in 1968, Slankis and I were discussing mycorrhizal research in North America. On the way to visit with J. W. Gerdemann at his lab, I asked Slankis what he thought of the idea of having a meeting of North American mycorrhiza workers. He agreed that this would be very worthwhile. I asked Gerdemann the same and he too agreed, if I would organize it. I said I would organize it, if he would host it. We agreed upon that and I went to work. Questionnaires were sent to all persons I had knowledge of in the United States, Canada, and Mexico who had at some time worked on mycorrhizae. This included early workers A. B. Hatch, K. D. Doak, A. P. Kelley, H. L. Mitchell, R. E. McArdle, and S. W. Wilde. The list came to approximately 50 persons. Of all who were invited only three, McArdle, Kelley, and J. R. Schramm sent regrets that they would not be able to attend, but were enthusiastic and supportive.

A program was developed to educate each other and to give us a base line for evaluating the research status of mycorrhizae in North America. We met at the University of Illinois in 1969.

Our program started with taxonomy. Alexander H. Smith presented a paper on the organisms we were working with in the higher Basidiomycetes. We heard other papers on other topics by invited speakers. All conference participants were invited to contribute papers, which most did. The papers were compiled in the Proceedings of the First North American Conference on Mycorrhizae and published by the United States Forest Service in 1971.

Limiting attendance at the 1st NACOM to those in North America was a deliberate effort to give North American mycorrhizasts the opportunity to meet each other to express their interests, and to help each other understand the problems that we faced. We thereafter became international from the second meeting to the present. I believe that the efforts expended in the organization of these two groups in particular, the IUFRO Mycorrhiza Group and the North American Conference on Mycorrhizae, have been vitally important in the development, expansion and increased credibility of research on mycorrhizae. It is impossible now for meetings such as this to be a forum for all who wish to present oral papers and smaller regional meetings might be worthwhile. However, I think all of us appreciate the benefits derived from the personal contacts at these conferences.

As an outgrowth of our communication and meetings, the Directory of Mycorrhizasts, of which the second edition has appeared for this meeting, is a very valuable asset and we are indebted to Valentin Furlan and André Fortin and their colleagues for developing and issuing this Directory.

Comment

These few highlights on the history of ectomycorrhizal research have been related from a personal point of view. I have known and enjoyed a warm friendship with most of the principals. Progress in ectomycorrhizal research has been often sporadic and slow. Lack of financial and administratiave support has been a difficult barrier for many to overcome and often has forced excellent mycorrhizal researchers into other pursuits. With the mounting interest in mycorrhizae now apparent, the future looks more promising. Hopefully the long intervals of quiescence between major discoveries will no longer prevail.

ENDOTROPHIC MYCORRHIZA (1885-1950): THE DAWN AND THE MIDDLE AGES

By

Barbara Mosse

The organisers of this symposium did not want a dry recitation of chronology; they wanted the people, the development of ideas and controversies brought alive. It has been interesting to do just that, and I hope that I can share with you some of the discoveries I made and the enjoyment I have had. This account, then, is divided into three sections; the history and the people that made it, the theories, and the chastening evidence that few things are new.

The History

Anyone interested in the early history of mycorrhizal studies cannot do better than to consult the account given by Dr. M.C. Rayner, published as a series of eleven chapters in the New Phytologist of 1926/7. More will be said about her reseaches later, but I think she must be credited with starting the tradition of publishing mycorrhiza papers in that journal (Rayner 1916). She divides the history of mycorrhiza research into an early period from 1840-1880, a second period from 1880-1900 and a modern period, 1900-1925.

The early period was concerned mainly with studies of orchids, particularly the saprophytic Neottia nidus-avis and an ericaceous saprophyte Monotropa hypopitys. For most people, however, mycorrhiza research begins with Frank, and it is indeed the centenary of his classic paper of 1885 that the 6th NACOM Conference is commemorating. Although it seems that Frank was by no means the first to observe and describe the regular fungal infection of temperate tree roots, he invented the name mycorhiza (with one r). His interests included a wide range of other nonpathogenic root infections that he grouped under the name "endotrophic mycorrhiza" in which the fungus entered into the cortical cells of the higher plant root.

The term "endotrophic mycorrhiza" thus covered a range of disparate phenomena from vesicular-arbuscular to orchidaceous and ericaceous mycorrhiza, as well as the widespread but little understood root infections of ferns and liverworts. To some extent all these studies inevitably follow a logical progression from descriptive to ecological, to experimental (isolation of the fungi and study of their effects on the host) and finally physiological (study of the mechanism of such effects and of fungus physiology). If one constructs a table showing this development for the various types of endomycorrhiza, it becomes evident (Table 1) that seniority in endomycorrhizal studies can fairly be claimed for the orchids.

Orchid mycorrhiza. The foundations for a proper understanding of orchid mycorrhizas were laid by the researches of Noël Bernard, a Frenchman, and of Hans Burgeff, a German. Bernard (1904) set himself three objectives: to germinate orchid seeds aseptically, to isolate and identify the fungi concerned and to compare the behaviour of infected and uninfected seedlings of different orchid species. He was thus the first to demonstrate experimentally the need for infection of the protocorm in normal development of many orchid seedlings. His experiments with Cypripedium showed that, unless infection occurred, the embryo accumulated much starch but expanded little, failed to develop chlorophyll and finally died. When inoculated with Rhizoctonia sp. isolated from infected seedlings, the fungus entered the embryo via the suspensor and invaded adjacent parts. After only ten days, digestion of the fungus began near the center of the infected zone, while the fungus continued to spread in the peripheral cells. The starch deposits disappeared, absorbing hairs and an apical meristem developed, and the first green leaves appeared after three months. Expanding his observations to other orchid species, Bernard found three different kinds of behaviour; without infection the seedlings failed to germinate, germinated but did not develop further, or rarely (eg. Bletilla) developed normally. Later, Bernard (1909) postulated a line of ontogenetic development within the Orchidaceae from independent to mycotrophic germination; the latter he considered the more highly evolved state. Bernard perfected a method of extracting the endophyte manually from infected peripheral cells and studied specificity in a series of cross-inoculation experiments with different orchid species and endophytes of different provenance. Three types of reaction occurred: the fungus behaved as a parasite and killed the seedlings; it developed a symbiotic relationship dependent on the control of fungal spread through limited intracellular digestion; or digestion was uncontrolled, the fungus was killed and seedling development arrested. Such seedlings could not thereafter develop a symbiotic relationship even with a more suitable endophyte, i.e. they had acquired some degree of immunity to infection. A heat-sensitive fungicidal substance could be extracted from the live but not from dead tubers of Ophrydeae (Bernard 1911). Later Burges (1939), using a microdissection technique, was able to extract a fungicidal substance from tubers and cells in which the fungus was undergoing digestion. Bernard (1909) also found that endophyte cultures maintained for more than a year on synthetic media lost their effectiveness, and that nonsymbiotic germination could sometimes be induced by organic substances. Noël Bernard died only twelve years after the publication of his first paper. Clearly he was a giant among researchers. He worked first at Rouen and later at the Institute Pasteur in Paris. Doubtless his view of mycorrhiza as a basically pathogenic phenomenon, with phagocytosis of the invading parasite, degrees of virulence of the invading microorganism and acquisition of immunity by the host, was to some extent influenced by his medical surroundings and colleagues.

By contrast Burgeff, the other great researcher of orchid mycorrhiza, who began as assistant of E. Stahl in Jena and enjoyed a working life of 50 years, believed in the essentially symbiotic (mutualistic) nature of mycorrhizal infection.

He confirmed and expanded Bernard's observations for a large range of orchid species and produced some superb pictures of the anatomy of the infecton (Burgeff 1909). He studied the enzymes of the fungi and searched for an explanation of the curious behaviour of starch reserves in the embryo, which he believed to be central to the problem of symbiotic germination. In 1936 Burgeff concluded that the accumulation of starch, particularly on carbohydrate-rich media, was due to the absence of some growth promoting factor of the vitamin B group in the developing seedling. This factor was present in yeast extract, in extracts of all strains of Rhizoctonia mucoroides and in various plant decoctions. Finally Schafferstein (1941) showed that, in addition to some other vitamins, nicotinic acid regularly and markedly stimulated germination.

Of most practical value to orchid growers was the work of Knudson begun in 1922. He elaborated various media for asymbiotic growth of orchids and eventually grew a Cattleya sp. aseptically to flowering in a succession of agar media (Knudson 1930).

By 1950, then, the essential features of orchid mycorrhizas were well known: the function of infection in seedling development, the requirement for vitamins and growth factors as well as carbohydrate, the range of species dependence on symbiosis and the range of host:fungus specificity. Many orchid mycorrhizal fungi had been cultured and their physiology and pathogenic potential in other plants were well known (see Harley 1959). By contrast, studies in other groups of endomycorrhiza had passed little beyond the descriptive and ecological stage.

Ericaceous mycorrhiza. The first good account of infection in the ericaceous Monotropa hypopitys, an herbaceous saprophyte found in woodlands, was given by Kamienski in 1881. He described the haustorium-like intracellular infection and the investment of fungal hyphae around the roots, thus predating to some extent Frank's (1885) description of ectomycorrhizal roots. Kamienski thought that the funus involved was probably the same as that infecting nearby trees and that the achlorophyllous Monotropa might indeed obtain nourishment indirectly from these trees via the common root inhabiting fungus. This view was later supported by work of Björkman (1960). In 1907 Ternetz isolated five picnidia-forming fungi from the roots of five ericaceous species. She assigned the fungi to the genus Phoma and claimed that they fixed small amounts of atmospheric nitrogen. For many years thereafter species of Phoma were considered the mycorrhizal fungi of the Ericaceae. In 1911 Rayner started a series of investigations on Calluna which continued during the next 15 years. Her name is probably best known in relation to the long-standing controversy concerning systemic and seed infection by mycorrhizal fungi in Calluna and other species. She summarized her findings as follows (Rayner 1926): the young roots of Calluna are extremely fine with only a single layer of large cortical cells. Under normal conditions each cortical cell encloses a dense branch system of mycelium directly connected with hyphae on the external surface. Some of these hyphae are fine and hyaline, others brownish and coarse. Throughout the growing season, but varying with time of year, age of root and external conditions, the infected cells show active intracellular digestion associated with the usual signs of increased cell activity. Rayner believed that another fungus existed in a more or less attenuated form throughout the whole plant and was present also in the seed coat. She considered (Rayner 1915) that the stimulus produced by this fungal infection was necessary for normal seedling development in Calluna, whereas the mycorrhizal infection of the root was a separate phenomenon, not obligatory under culture conditions but normal in nature. Christoph (1921) and other subsequent researchers disputed the presence of, or need for systemic infection, and their view has since prevailed.

In 1924 Rivett described another form of mycorrhiza in Arbutus unedo, more reminiscent of typical ectomycorrhizal infection. This tree formed sporadically infected long roots and short roots or tubercles, similar to the dichotomously branched roots of Pinus. The tubercles had a fungal mantle and intracellular peloton-like digestion occurred in the cortical cells.

By the midcentury (1950) the only certain information about ericaceous mycorrhizas was that they were extremely variable, ranging from something resembling orchid mycorrhizas to something more like typical ectomycorrhizas. It was thought that the infection was probably important, if not essential, in the achlorophyllous species.

Pteridophyte and Hepatica mycorrhiza. It was known by 1950 that many Pteridophytes had mycorrhizal roots, but a complete anatomical study of the group was not available till 1957 when Prof. Boullard, another Frenchman from Rouen, published his review of "Mycotrophy in Pteridophytes, its frequence, characteristics and significance". Many have typical vesicular-arbuscular mycorrhizas. The same is true of the liverworts, whose mycorrhizal systems were studied by Marianne Stahl (1949), a student of Burgeff.

Vesicular-arbuscular (VA) mycorrhiza. As early as 1900 it was well known that by far the most widespread mycorrhiza was the vesicular-arbuscular type. Their first description is usually attributed to a famous French mycologist Dangeard, who described them in two short articles as "Une maladie du peuplier" (1896) and "Le Rhizophagus populinus, Dangeard" (1900). Dangeard really studied sexuality in fungi and described the cytology of clamp connections, which in French are known as "Dangeardie". He must have been a quite remarkable person, filling up single handedly vols. 1-5 of "Le Botaniste" comprising over 1000 pages, and at least half of vols. 6-9. He was also the editor of that journal. Dangeard (1900) produced very simple, accurate pictures of the infection in poplar roots, including both

arbuscules and vesicles and clearly illustrated the multinucleate condition in vesicles and hyphae. He also caused a certain amount of subsequent confusion by naming the fungus "Rhizophagus" (devourer of roots) and assigning it to the Chytridiaceae, on no very good evidence. The name persisted for a long time and was used as late as 1963 by Greenall to describe a new species, Rhizophagus tenuis. Priority in the description and extensive studies of distribution of VA infections really belongs to Schlicht (1889), a student of Frank, and to Janse (1896), who drew attention to the widespread nature of such infections in tropical plants. Janse first named the vesicles "vesicule"; (he wrote in French) and was also the first to use KOH to stain infection in entire coffee roots. Infected roots went red. Schlicht's (1889) descriptions and ecological observations were remarkably detailed. He stated that the fungus always entered from the soil, caused no change in root anatomy, was rarely septate and contained many oil droplets, that it regenerated within the cells into cauliflower-like shapes which darkened with age, and that infected roots appeared vigorous and healthy. He observed that the fungus spread towards the root tip, was confined to the primary cortex, particularly the inner layers, and that the main roots of Ranunculus were not infected. He concluded from his ecological survey that water plants were not infected although some plants in temporarily flooded situations were, and that plants in humus-rich soils were often mycorrhizal. He noted that cultivated plants could be mycorrhizal but were rarely so when grown in garden soil. Finally, Schlicht considered that several fungi probably caused such infections, a correct view widely disbelieved half a century later. Important also about that time were the extensive studies of Gallaud (1904), yet another Frenchman, who invented the term "arbuscule" and beautifully illustrated their different developmental stages. Previous, and to some extent subsequent observers believed that the drupe-like structures they called "sporangioles" represented some specialised kind of spore, whereas Gallaud correctly regarded them as degeneration stages of arbuscules.

Subsequent investigations of VA mycorrhiza had three objectives: 1) to study the occurrence of VA mycorrhiza in different families and species (Peyronel 1924, Asai 1934) in the same species at different locations (White 1929, Lihnell 1939, Neill 1944, Winter and Birgel 1953) and in various crop plants (Jones 1924, Rayner 1939, Winter 1951); 2) to compare the growth of mycorrhizal and nonmycorrhizal plants and the effects of manurial treatments on mycorrhizal development (E. Stahl 1900, Demeter 1923, Reed and Frémont 1935, Asai 1943, 1944; Laycock 1945, Johnston 1949) and 3) to identify and culture the endophytes (Peyronel 1923, 1937; Butler 1939, Magrou 1946, M. Stahl 1949).

All surveys confirmed the widespread occurrence of VA infection with the notable exception of Chenopodiaceae, Centrospermae and a few smaller families (see Gerdemann 1968). In spite of many attempts to link incidence with particular soil conditions, notably humus content, no clear pattern emerged except the scarcity of infection in very fertile soils.

All experimental evidence of growth differences between mycorrhizal and nonmycorrhizal plants remained inconclusive, because the only inoculum was natural soil or infected roots growing in such soil. Quite often the inoculated plants did not become mycorrhizal. From the 1930s onwards the consensus of opinion was that the fungi were probably mild pathogens. Markedly at variance with this view were the results of Asai (1943, 1944). He grew a range of plants in autoclaved soil with and without a small inoculum of garden soil. Many of the plants that did not receive the garden soil inoculum stopped growing after a good start. Among such plants were both normally endo- and ectomycorrhizal species. Inoculated plants showed considerable variation in response; those benefitting little were also the least infected. Only mycorrhizal plants benefitted from added fertilizer. A much smaller group of plants, belonging mainly to the Polygonaceae and Centrospermae, grew well without inoculation, indeed tended to be somewhat larger than the inoculated ones. Asai isolated a species of Fusarium from infected grass roots. With this isolate he claimed to have produced mycorrhizal infection in other grasses (Panicum, Penisetum and Setaria) but not in other species, thus preceding Russian claims that a Fusarium sp., isolated from mycorrhial roots, produced infection and growth improvements in Gramineae. From an ether extract of the Fusarium, Asai obtained a crystaline substance that somewhat stimulated root growth. I am giving these results in some detail, because Asai, a Japanese, published his papers in the Japanese Journal of Botany in an almost incomprehensible German. In a second paper Asai (1944) reported that without garden soil inoculum and subsequent mycorrhizal infection legumes did not nodulate in the autoclaved soil, although rhizobia had been added. Asai's results were largely discounted by the scientific community at the time. They were explained on the basis of soil toxicity induced by autoclaving, which kept the plants generally small, and by the detoxifying effect of microorganisms in the garden soil inoculum. With hindsight it is obvious that this explanation was not tenable. But, sterilization-induced toxicity was fashionable at that time. I have heard Prof. Gerdemann remark that, if his work did nothing else than to nail the myth of widespread soil toxicity following sterilization, he would be content.

Practically every one who ever took an interest in VA mycorrhiza has tried to culture the endophytes. A range of septate mycelia including Fusarium, Pythium, Rhizoctonia and others were isolated from surface sterilized roots (see Harley 1950). None of these isolates produced proper infection when back-inoculated under sterile conditions. A few reports are worth more detailed consideration. In 1923 and again in 1937 Benjamino Peyronel, working in Rome and later in Turin, reported finding hyphal connections between fruitbodies of Endogone vesiculifera, E. fuegiana, and a form of E. macrocarpa and the endophyte in roots of various plants growing in the alpine region of the

Piccolo San Bernardo. Then, as now, some mycorrhiza enthusiasts have found nice places to conduct their researches. Butler (1939), in a very well informed review, cited this and other strong circumstantial evidence supporting this identification of VA endophytes. Sir Edwin Butler was a highly respected British mycologist who, after his retirement from the Colonial Service, devoted some time to the study of VA mycorrhiza. He had been impressed by their prevalence in cotton in the Sudan.

Three attempts to culture VA endophytes showed some measure of success. Magrou (1946), a Frenchman, obtained limited hyphal growth from surface steriized Arum roots in hanging drop cultures. Optimum growth occurred at pH 6.6 and was stimulated by egg albumin and peptone, notably the vitamin B (nicotinic acid) and aneurine components, predating some more recent findings of Hepper (1979). He also noted that hyphal regeneration from infected roots was best when roots were at the stage of vesicle formation, predating some results of Biermann and Linderman (1981). Marianne Stahl (1949), at the University of Würzburg infected young Marchantia gemmae growing in sterile sand from washed, mycorrhizal thalli across a distance of 3-4 cm. She also obtained considerable hyphal growth from thalli suspended in sterile rain water, again recording a pH optimum between 6.0-7.5, stimulation by egg albumin and 1% $CaCl_2$. Perhaps most intriguing are the results of J.T. Barrett (1947) who claimed to have cultured a VA endophyte, which he persisted in calling Rhizophagus. Starting with unsterile mycorrhizas, he used small pieces of hemp seed as a bait and, after several transfers to fresh hemp seed, obtained a fungus that grew on normal fungal media. I have myself seen these cultures in Prof. Barrett's laboratory in the Plant Pathology Department at Berkeley. The fungus had a coarse, aseptate mycelium and formed many vesicle-like spores. The technique has been repeated by Prof. Gerdemann but, so far as I know, has never succeeded when aseptic mycorrhiza roots were used as the starting point. Prof. Barrett claimed that he could obtain typical VA infection from inoculation with this fungus, but these results were somewhat dubious and were never published. Prof. Barrett told me that, in order to re-isolate the fungus from mycorrhizas produced by back-inoculation, it was necessary to pass again through the hemp seed stage, suggesting a discontinuous saprophytic and symbiotic phase. In hemp roots VA infection is exceptionally vigorous. Like Butler (1939) and Neill (1944), Prof. Barrett took up this work after his official retirement.

Theories

Although the earliest investigators often had little experimental evidence, this in no way prevented vigorous and lengthy discussion on the probable significance of the mycorrhizal phenomenon. There were two basically opposed views: that the plant benefitted in some way from the association, or that the fungi were mild, or even virulent pathogens over which the plant exerted some mechanism of control. Although well aware of their beneficial effect on early seedling development in orchids, Bernard tended to regard the majority of mycorrhizal associations as instances of controlled parasitism. Gallaud supported this view for VA mycorrhiza, largely on histological grounds, considering that the cells showed some evidence of resistance to invasion, passively by depositon of a membrane around the arbuscule and actively by arbuscule digestion. O'Brien and M'Naughton (1928) in Scotland and two Canadians, Truscott (1934) and Koch (1935), even considered VA mycorrhiza to be the cause of various root rots, if not directly, then as forerunner of other, more pathogenic fungi. On the other hand Frank and his followers held that mycorrhizal infection was nutritionally beneficial to the host. Opinions differed on how such effects might arrise. Originally Frank postulated some ability of mycorrhizal fungi, or the mycorrhizal root, to utilize organic substances in the soil that were unavailable to nonmycorrhizal roots. E. Stahl, a student of Frank, concluded after an exhaustive study of the habitats and anatomy of a large range of mycotrophic and autotrophic plants, that mycotrophy was in some way related to difficulties in nutrient uptake. Believing this to be linked to water uptake, he thought that plants with a low water throughput, related to restricted root systems (notably thick, fleshy roots) and low transpiration rate (i.e. leaves with thick cuticles and no hydathodes) were more dependent on mycotrophic nutrition. Owing to low transpiration such plants also had a low ash content. Stahl also repeated observations first made by Frank and widely accepted at that time, that mycorrhizal plants were commmon in soils lacking nitrate and did not themselves contain any, whereas nitrate, as indicated by the diphenylamine sulphate test, occurred in the same plants when they were nonmycorrhizal. Because mycorrhizal infection was often abundant in high organic matter soils, beneficial effects were thought to arise from utilization of complex organic substances of a nitrogenous or humic nature. Ericaceous endophytes were thought capable of fixing small amounts of atmospheric nitrogen. In general nitrogen was thought to be the important factor in mycotrophic nutrient uptake. One problem of that period was that explanations were sought that would cover the whole range of mycorrhizal phenomena. It is interesting how little specialization there was. Thus Frank worked on ecto-, orchid, ericaceous and VA mycorrhizas, Burgeff on orchids and pteridophytes, Bernard on orchids and VA's, Gallaud on VA, hepatic and ericaceous mycorrhizas, Magrou on VA's and orchids, and Knudson on orchid and ericaceous mycorrhizas. Also the problem of achlorophyllous plants obscured the issue, as attempts were made to correlate development of the mycorrhizal habit with saprophytism.

As the 20th century progressed, investigators of VA mycorrhiza became increasingly convinced that, contrary to ectotrophic and orchid mycorrhizas, VA endophytes could not fulfill any major role in plant nutrition because live hyphal links between the soil and root-based mycelium were insufficient for a major nutrient transfer. Any putative benefit the plant might derive resulted, it was thought, from arbuscule digestion and discharge of fungal material of a

mainly proteinaceous nature (Eiweiss-substanz) and/or lipids (McLennan 1926) into the plant cell. The internal mycelium, well supplied with carbohydrate from diminishing starch reserves in the root, would take up from the soil corresponding amounts of nitrogen needed for balanced fungal growth. This might represent a small net gain of nitrogen to the plant. Reed and Frémont (1935) observed increased arbuscule development in roots of citrus given farmyard manure compared with those given equivalent amounts of artificial fertilizer. If arbuscules were the main source of benefit to the host, it was argued, then organic manures might indeed improve the efficacy of VA infection. Bruno Peyronel (1942), confirming earlier observations of his father Benjamino Peyronel (1940a, 1940b) concerning the relationship between light, humus and VA infection, concluded that in liverworts the dependence of mycorrhizal infection on light intensity was less pronounced in humus-rich than in humus-deficient soils. Mainly due to the work of Dr. Rayner on ectomycorrhiza at a forest nursery at Wareham, England, the benefit of composts came to be linked to their effects on mycorrhizal infection. Thus, mycorrhizal studies became involved in the much older and still continuing controversy of organic manures versus artificial fertilizers. This did little to raise their reputation in the scientific community. The ill defined advantages that many practical plant growers attributed, and perhaps still attribute, to composts and organic manures, were thought to be mediated through mycorrhizal infection. Sir Albert Howard's (1940) book, "An Agricultural Testament", based on his experiences with composting in India, finally sealed the fate of VA studies for several decades. They became the "mal aimee des microbiologists" (Bertrand, 1972) and have only partially recovered from this even now. In the latest edition of the Encyclopaedia Britannica "mycorrhiza" is not even listed separately. They merit seven lines in a six page dissertation on Mycota by Alexopoulos. He describes them as "a mild form of parasitism that in many instances verges on mutualism".

Two other theories are of some historic interest. Because so many attempts at isolating VA endophytes ended up with septate mycelia, often of the Rhizoctonia type, and because of occasional septation and the apparent dimorphism of the mycelium in the root, Peyronel (1923) considered the possibility of a "dual infection" by two separate fungi, a Phycomycete, probably a species of Endogone, and a Rhizoctonia. Later he regarded the Rhizoctonia as a secondary invader. A regular intracellular infection of Lolium seed was described by McLennan (1920) in Australia. The endophyte was present in the embryo sac immediately after fertilisation, developed at the expense of the nucellus, and was eventually digested as the embryo developed. In 1926 McLennan described in addition a classical VA root infection in Lolium. The theory of "dual infection" in VA mycorrhiza has some interest in view of more recent claims by Williams (1984) concerning the regular isolation from excised vesicles of a "companion fungus" with beneficial effects on plant growth.

Perhaps even more remarkable was a view that tuberisation was in some way related to mycorrhizal infection. Bernard (1902) proposed this for the Ophrydeae, and E. Stahl considered that plant species producing bulbs and tubers were especially prone to mycorrhizal infection. Bernard suggested that a brief period of growth and differentiation with no infection alternated regularly with a longer period of tuberisation following infection. The tubers themselves remained uninfected because of their fungicidal properties. Magrou and Magrou (1940) attempted to extend this to potatoes. They noted that potatoes growing under alpine conditions were strongly mycorrhizal, whereas cultivated varieties were not. This they regarded as varietal adaptation. Among progeny of Solanum dulcamara two types of seedlings appeared, mycorrhizal with tubers and nonmycorrhizal without.

Few things are new

In this review of the history of endomycorrhiza I will surely have omitted someone's favorite researcher and work. For this I apologize.

In trying to arrive at some overall view of the period, I am struck by three things. The period around the turn of the century was one of beautiful observation, great enthusiasm and, particularly for orchid mycorrhiza, of great advances. The most important contributions to the study of endomycorrhizas in that period were made by continental Europeans, foremost by the French, followed by the Germans and Italians. Dr. Rayner was probably the first noteworthy Anglo-Saxon contributor to mycorrhizal studies. After this initial period of great developments, endomycorrhizal studies between the wars entered a stage that can fairly be compared to the dark Middle Ages, whereas ectomycorrhizal studies continued to advance. Perhaps recent times have somewhat redressed the balance.

It is also inescapable that much of what has been hailed as new discoveries in the upsurge of the post-1950's is in fact a rediscovery of what was said much earlier. I have tried to draw attention to this throughout this talk. Particularly the anatomical and ecological observations of the earlier period were extremely good, although recent observations, particularly use of the electronmicroscope, have perhaps put them on a more exact basis. Nevertheless, both Gallaud (1904) and Demeter (1923) observed that walls of soil hyphae contained one more layer than those within the root. Both Gallaud (1904) and Burgeff (1909) state that arbuscules are surrounded by a fine host membrane. I quote: "the penetrating hypha is immediately surrounded by a fine protoplasmic skin produced by the host cell." Another observation, yet to be rediscovered, is that there are special passage cells in the hypodermis, related to fungal entry and that mycorrhizal fungi degrade pectin, cellulose and the middle lamella (Demeter 1923). Certainly the relationship between soil fertility, root morphology and mycorrhizal infection was extremely well documented before 1950. The observation that mycorrhizas flourish on nutrient-poor soils, and that they are

particularly evalc in plants with thick, fleshy roots, few root hairs or otherwise reduced root systems, was made by Schlict (1889), Stahl (1900), Gallaud (1904), Petri (1908), Burgeff (1909), Demeter (1923) and others. It was summarized by Peyronel (1937) as follows: "Plants fall into two large groups according to their root development: a) those with thread-like, very thin roots with long and many root hairs are rather sparsely mycorrhizal while still in the active living state and b) those with thick, fleshy roots are usually strongly mycorrhizal. In mixed communities plants are usually more strongly mycorrhizal than in monoculture". "Mycorrhiza are often lost when plants are moved into monoculture or green houses" (Winter and Birgel 1953). One could multiply such examples without difficulty. Perhaps the best I have found is the remark in the preface of Dangeard's short, but famous paper describing Rhizophagus populinus. He says, "The preliminary note published (on the illness of poplars) was intended to be followed by a fuller account of the development and progress of the parasite. But, the assistance from the Ministry of Agriculture on which I had rather depended for the succesful conclusion of these studies was not forthcoming, and I therefore now present here the main facts as observed some years ago."

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Table 1. Historical Progression of Research on Endomycorrhizae and Endomycorrhizal Fungi.

Type	Descriptive	Ecological	Experimental[1]	Physiological[2]
Orchidaceous	Bernard 1902, 1904 Burgeff 1909	Burgeff 1909	Bernard 1903, 1904 Burgeff 1909, 1936 Knudson 1922, 1930	Bernard 1902, 1911 Burgeff 1909, 1936 Burgess 1939 Schafferstein 1941
Ericaceous	Kamienski 1881 Rayner 1915 Rivett 1924		Ternetz 1907 Rayner 1915, 1926/7 Christoph 1921	Ternetz 1907
Pteridophytes & Hepatics		Boullard 1957 Stahl, M. 1949		
Vesicular-arbuscular	Schlicht 1889 Janse 1896 Dangeard 1900 Gallaud 1904 McLennan 1920, 1926 Demeter 1923 Jones 1924 O'Brien & M'Naughton 1928 Reed & Frémont 1935 Koch 1935 Peyronel 1923, 1927 1937 Butler 1939	Schlicht 1889 Stahl, E. 1900 Petri 1908 Demeter 1923 White 1929 Asai 1934 Lihnell 1939 Peyronel 1923, 1940 Peyronel, Bruno 1943 Neill 1944 Winter 1951 Winter & Birgel 1953	Laycock 1935 Asai 1943, 1944 Magrou 1946(c) Barrett 1947(c) Johnston 1949 Stahl, M. 1949(c)	

[1] culture of endophytes (c) and effects on plant growth.
[2] physiology of mycorrhizal fungi and mycorrhiza.

VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI: 1950 TO THE PRESENT--THE ERA OF ENLIGHTENMENT

by
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Key words: History, Review, Phosphorus-uptake, Taxonomy, Methodology, Interactions, Axenic-culture.

Introduction

In the period 1950 to 1984, we advanced from knowing very little about the symbiosis of vesicular-arbuscular (VA) mycorrhizae to that of knowing considerable about the fungi involved and the association itself. We have taken VA mycorrhizae from the state of being a curiosity to that of being a potential valuable resource in agriculture. We have begun to open the world's eyes to the importance of a symbiotic association that was for decades slighted. We now have the research to substantiate the widespread nature and importance of VA mycorrhizae and we can see its potential in the future. Gerdemann was correct when he stated, "if (this association) has even a slight direct or indirect effect on plant growth, (its) economic importance is considerable".

The increase in number of researchers, research papers, and meetings are indicative of the increased interest in VA mycorrhizae. The number of researchers involved with VA mycorrhizae in the 1950's was less than 10. In the 1981 International Directory of Mycorrhizae Researchers, 319 individuals indicated a research interest in VA mycorrhizal fungi. In 1969 at the first NACOM meeting, 57 scientists were present while at this 1984 Sixth NACOM meeting approximately 300 scientists are present. In 1950 the book "Mycotrophy In Plants" by Kelly was published. Nine years later Harley's book "The Biology of Mycorrhiza" was published and his second edition appeared 10 years later. That represented a total of three books in the first 20 years of this period. In the last 13 years, seven books have been published in which a major portion dealt with VA mycorrhizae, and this does not include many other books with single chapters on VA mycorrhizae. Yes, we are having a much greater impact now than 34 years ago.

1950 to 1960: The Keys Are Found

Let us take a closer look at those early years to better understand how our "science" developed. Although VA mycorrhizae had been observed by many researchers, there was considerable debate over what fungus or fungi were involved. It was certainly "phycomycetous" for the hyphae were clearly nonseptate and hence many individuals thought the Pythium species, that were frequently isolated on culture media from mycorrhizal roots, might be the causal agent. It was not until 1953 that Barbara Mosse in England found the "key" to solve the mystery. She was able to establish a 'pure' pot culture of mycorrhizae on strawberry seedlings using spores of a fungus associated with strawberry roots in the field. Shortly thereafter (1955), Gerdemann in Illinois was able to establish VA mycorrhizae in pot cultures on several plant species using spores of another fungus from maize. Gerdemann used the wet-sieving technique, employed by nematologists, to extract spores of the mycorrhizal fungus from soil. Thus, in just a few short years fungi capable of establishing VA mycorrhizae on plants were isolated, a means of increasing them was determined, and a technique was adapted for removing the spores of these fungi from soil. The corner stone was laid for all subsequent studies on VA mycorrhizae.

There were others working with VA mycorrhizae during this period. Barrett at the University of California was culturing VA mycorrhizal fungi on media, or so he thought. I will say more about this later. Tolle and Hawker were attempting also to grow the fungus causing VA mycorrhizae from roots. Bayliss in New Zealand and Puess in Germany were demonstrating that VA mycorrhizae made plants grow better and increased plant phosphorus levels.

1960 to 1970: The Race Begins

The number of researchers and the reports on mycorrhizae began to increase rapidly during this decade. There were numerous reports of increased growth response of VA mycorrhizal plants compared with nonmycorrhizal plants. These were largely greenhouse pot studies using steamed, autoclaved, irradiated or fumigated soils. Even at this early stage there were a few others (Meloh, Holevas) who were ahead of their times and reported plant growth depressions with mycorrhizae. Researchers went to considerable effort in these early studies to establish that the mycorrhizal fungus and not associated organisms were causing the increased growth. Perhaps this explains why several attempts were made during this period to establish these fungi on a host gnotobiotically. Those working with pot cultures usually relied on spore washings or autoclaved roots to inoculate control plants without VA mycorrhizal fungi. However, Clark used killed spores, that were individually punctured with a needle, to inoculate nonmycorrhizal plants.

Several surveys to determine the extent of these fungi were initiated in this decade. It became obvious that several morphologically different fungi were associated with VA mycorrhizal fungi and the first species descriptions by Nicolson and Gerdemann were made. However, as late as 1962, Hawker reported that Pythium species were the causal agents of VA mycorrhizae.

Numerous individuals were demonstrating that VA mycorrhizae increased uptake of phosphorus from soils. In several of these studies, various rates of phosphorus applications, times of application and phosphate sources were utilized. There was some suggestion that VA mycorrhizal plants might be able to utilize insoluble forms of phosphate in the soil. Bayliss was developing his theory in regard to

the relative need of course-rooted (magnolioid) and fine-rooted (graminoid) plants for VA mycorrhizae.

In this decade, the first reports of adverse effects of pesticides and soil fumigants on mycorrhizal fungi were noted. Ultrastructure studies on VA mycorrhizae in roots were initiated and the first documentation of a VA mycorrhizal fungus lessening plant stress from a plant pathogen was reported.

By now the initial foundation for the research in the 1970's and 1980's had been laid. Since space does not allow a detailed review of the research in the 70's and 80's, only a summary of some of the major areas of VA mycorrhizal research will be presented.

Host Specificity and Mycorrhizal Dependency
(Is Their Effect Different?)

From the very early research of Mosse and Gerdemann, it was apparent that VA mycorrhizal fungi have extensive host ranges. We now know they are perhaps the most extensive host ranges of any soil-borne fungi. However, Mosse indicated that as early as 1958 Tolle reported some specificity among these fungi. Mosse and Hayman noted that indigenous mycorrhiza from several soils varied in the ability to induce a plant reponse in onion. In some, a good plant response occurred, in others no response was obtained. In another test, different species of VA mycorrhizal fungi were shown to differ in the ability to induce a growth response in Bahiagrass (Paspalum notatum). Gilmore observed that species of mycorrhizal fungi differed in their ability to aleviate Zn deficiency in peaches. Others have obtained similar results on a number of hosts. Obviously, the host-fungus reaction is specific despite the broad host range of the fungal symbiont. The ability to colonize a host does not insure an increased growth response.

Several factors involving soil edaphic and environmental conditions and the innate properties of the fungus can influence this specificity. Properties of the host also are probably involved. When Bayliss noted different responses among hosts in regard to inoculation with VA mycorrhizal fungi, he suggested that root geometry had an impact on plant response. He noted that plants with course root systems and sparse root hairs (magnolioid type) were more dependent on mycorrhizae for normal growth in low phosphorus soils than finely rooted plants with numerous root hairs (graminoid type). There have been several exceptions found to this hypothesis, thus it cannot be applied universally, but it probably applies in many instances.

Gerdemann proposed a means of evaluating the mycorrhizal dependency of a host. Menge expressed it numerically as the ratio of the weight of a mycorrhizal plant compared to a nonmycorrhizal plant converted to percent. This method of calculating mycorrhizal dependency has been utilized primarily in pot and greenhouse studies. Plenchette et al. has modified this method for use in field studies.

Uptake of Phosphorus
(A Pipeline in the Soil?)

If there is one thing established beyond a doubt with VA mycorrhizae, it is that they increase uptake of phosphorus from the soil. Some would suggest that all the plant effects by VA mycorrhizal fungi are the result of increased phosphorus uptake. Shortly after determining which fungi cause mycorrhizae, it took little time to demonstrate mycorrhizal plants acquired greater concentrations of phosphorus than nonmycorrhizal plants. Using radioactive isotopes of phosphorus, it was shown early that phosphorus was absorbed more in mycorrhizal plants than in nonmycorrhizal plants.

But what about the nature of this increased phosophorus uptake? Murdoch et al. and Daft and Nicolson using phosphorus sources of various solubilities determined that the growth difference between mycorrhizal and nonmycorrhizal plants was greatest with relatively insoluble forms of phosphorus. This suggested that VA mycorrhizal fungi may have the ability to solubilize phosphorus not available to the plant. Studies with ^{32}P labelled exchangeable phosphorus in the soil (Sanders and Tinker; Hayman and Mosse; Mosse et al.) showed rather conclusively that both VA mycorrhizal and nonmycorrhizal plants obtained their phosphorus from the same sources in the soil. Rhodes and Gerdemann demonstrated that hyphae from VA mycorrhizal plants were able to absorb and translocate phosphorus from up to 8 cm from the root. The calculated rate of phosphorus transfer in the hyphae indicated that the movement of phosphorus was above that explainable by diffusion alone. In addition, Cox and Tinker suggested an active transfer of phosphorus into the host in the arbuscle. According to Tinker and Gildon, mass flow and protoplasmic streaming offer a full explanation of the high rates of phosphorus translocation in VA mycorrhizal plants.

There are indications that VA mycorrhizal fungi are also involved with the translocation of other elements, including calcium, zinc, copper and sulfur. Several researchers have shown reduced Zn and Cu deficiency symptoms on mycorrhizal plants but such effects may have been obtained indirectly from increased growth resulting from increased phosphorus absorption by the mycorrhizal plants. However, Gildon and Tinker recently clearly established that in the presence of nonlimiting phosphorus levels in soil, VA mycorrhizal plants in copper limited soil significantly increased plant growth and copper concentrations in the plant while phosphorus applications had little effect.

Methodology
(How Many Ways Can You Do It?)

Advances in most fields of science are coupled to advances in methodology. Research with VA mycorrhizae is no exception. As I already

indicated, the methodology related to establishing pot cultures and wet-seiving in the 1950's were key to establishing the extensive research in the 60's and 70's. One of the most difficult tasks is that of estimating accurately the extent of VA mycorrhizal fungi in the plant root. There have been many methods developed for this purpose, most of which depend upon the adequate clearing and staining of roots to detect the fungus. The clearing and staining procedure developed by Phillips and Hayman is perhaps the most widely accepted. There have been several visual methods developed for assaying mycorrhizae in roots. Adaptations of the line intersect method (Giovanetti and Mosse), which provides estimates of percent mycorrhizae and also root length, are widely used. Chemical procedures are perhaps more quantitative than visual procedures but they are not useful in estimation of mycorrhizae in field grown plants. Although there have been attempts to standardize a method for assaying root mycorrhizae, at present it is usually not possible to make direct comparisons between researchers in this regard. For this reason, there are probably arguments between researchers over which method is most rapid, accurate and useful.

There are many methods used to extract spores from soil to estimate their numbers per unit of soil. The use of sedimentation, flotation and centrifugation are employed in removal of spores from debris but most methods primarily use wet-seiving procedures for initially removing spores from bulk soil. Centrifugation in sucrose is perhaps the most commonly used procedure for obtaining spores relatively free of detritus.

The application of the most-probable-number technique to estimate the number of viable propagules of VA mycorrhizal fungi in soils has proven useful. Although this procedure is far more accurate than spore counts, there are indications that several factors (Wilson and Trinick) can influence the results and perhaps a standard procedure would be best for making comparisons from one study to another.

Interactions With Other Microorganism
(Do They Bother Other Microbes?)

There are many reports on the interactions of VA mycorrhizal fungi and microorganisms causing plant dieases. Most of these have dealt with soil-borne or root-infecting microorganisms (primarily fungal pathogens or parasitic nematodes) and frequently the results are in apposition to each other. Some reports indicate a decrease in disease intensity with VA mycorrhizal fungi while others indicate the reverse. Perhaps a portion of this controversy revolves around the fact that different host-mycorrhizal-pathogen systems have been evaluated and no standard means of comparison was used. The degree of susceptibility of the host to the pathogen, the form and number of propagules of the pathogen, the method of inoculation, and the species of VA mycorrhizal fungi used can all affect the results. Few comparisons have been made in natural soils and few have been evaluated in the field. Many of the positive effects of VA mycorrhizal fungi on reducing plant stress from pathogens are probably simply indirect effects of increased phosphorus levels in the mycorrhizal host. Few evaluations have had adequate phosphorus level controls. As in other areas of VA mycorrhizal studies, there is room for more definitive studies in this area.

A few reports have been noted with free-living bacteria in the soil, such as nitrifying and P-solubilizing bacteria and VA mycorrhizal fungi. In these instances, the presence of the bacterium with the VA mycorrhizal fungus increased plant growth compared to the use of both separately. In the case of Azotobacter, the bacterium and VA mycorrhizal fungus had positive effects on each other, both stimulating the other. In a recent report of Ames et al. only one of five species of bacteria that were monitored were increased by mycorrhizae. The others were generally the same population in the rhizosphere of mycorrhizal and nonmycorrhizal plants.

Perhaps the most studied and well documented interaction is that between Rhizobium spp. and VA mycorrhizal fungi. In soils with low amounts of extractable phosphorus, VA mycorrhizal fungi have been shown repeatedly to effect nodulation and nitrification in legumes. Recently, nitrate reductase activity was also shown to be affected by VA mycorrhizal fungi. This stimulation is mainly an indirect effect of the mycorrhizae on the Rhizobium sp. through providing adequate phosphorus levels for normal Rhizobium activity (2 to 3 times higher phosphorus levels are required in nodules than in other plant tissues). Although the effect of mycorrhizae on nodulation occurs within a few weeks, a higher level of applied phosphorus is required to compensate for the mycorrhizal effect on nodulation and nitrogen fixation than is required for the mycorrhizal effect on plant growth.

Taxonomy
(How Many Species Can There Be?)

Taxonomists are a despicable group. They are obsessed with changing things and naming something new. What could have been a small group of organisms forming VA mycorrhizae with plants (one genus and only a few species) has become a tangled web. Tom Nicolson and Jim Gerdemann were the first of those to initiate the confusion when they found several obviously different fungi capable of producing mycorrhizae. There were others who from the beginning thought that there was no basis for naming these different fungi as new species, but that didn't prevent them from being described. Then Gerdemann joined forces with Trappe and taxonomy of the Endogonaceae got even more complicated. We than had several genera of fungi to contend with.

This then opened the door to a whole host of individuals that began to taxonomize. New species were described at a tremendous rate and a new genus was added. From only a few species

in 1970, we now have or will shortly have over 100 species of fungi that form VA mycorrhizae. This makes it difficult for the non-taxonomist, to say the least.

Why did these taxonomists do this to us? Simply because there really were many different fungi that could form VA mycorrhizae with plants. It was necessary to characterize and describe them adequately so other researchers could recognize them and could also determine when someone else was working with the same fungus.

Will it get better in the future? I am certain there will continue to be many more species described, but the most common ones in the world will remain those with which we are most familiar. As new species are described it will be the responsibility of taxonomists to also provide the keys and means for separating them from the existing species and to develop new methods of identification.

Axenic Culture and Spore Germination (Why Can't We Grow Them Alone)

From the very beginning, individuals have attempted to axenically culture VA mycorrhizal fungi without the host. Only Barrett reported success in culturing these fungi from several hosts, but unfortunately no one else was able to repeat his work, even when using similar media and Barrett's own cultures. Subsequent attempts by numerous individuals to grow these fungi in axenic culture without the host have all failed. No one has been able to transfer the hyphae from a germinated spore and obtain independent and extensive new growth. The result of these many attempts has been a profusion of papers on factors affecting spore germination and germ-tube growth. Many factors influence spore germination and germ-tube growth and several of these factors have been summerized (Siqueira et al.). Recently growth of hyphae detached from their spores on culture media (Hepper) and apparent growth of these fungi in soil without the host (Warner and Mosse) has been reported. In spite of the failure to grow these fungi asymbiotically, I am confident that in the near future someone shall succeed, just as they have with other obligate fungi.

Axenic dual culture of both mycorrhizal symbionts has been successfully accomplished by several researchers on a variety of complex culture media. Plant growth and mycorrhizal development are frequently restricted in many of these systems. Other systems for axenic dual culture have been devised using sand or soil as the medium and these have provided more normal growth of both symbionts.

Pesticides and VA Mycorrhizae (Are They Really That Sensitive?)

Since the late 1960's, it has been apparent that many modern pesticides can have serious effects on VA mycorrhizal fungi. The mysterious "stunting" that occurred on a number of crops after soil fumigation with methyl bromide and other general soil funigants was finally solved. The plants, obviously very mycorrhizal dependent, were slowly starving to death in the absence of mycorrhizae even in the midst of adequate levels of most nutrients. Several foliar and soil fungicides can reduce the incidence of VA mycorrhizal fungi (e.g. PCNB, Thiram, Botran). Systemic fungicides are generally more damaging to mycorrhizal fungi (e.g. benomyl, thiabendazole) than nonsystemic fungicides but some systemic fungicides have little or no effect (e.g. ridomil, terrazole) on VA mycorrhizal fungi. Although not as deleterious, some reduction in mycorrhizae has occurred with nematicides and herbicides although in many instances some are actually beneficial to growth or reproduction of mycorrhizal fungi.

Finale

I think we can be proud of our accomplishments in the past 35 years. We have learned much about VA mycorrhizae. Much of the research thus far has been accomplished in the greenhouse or laboratory. We are still not commercially using these fungi to any large degree in the field. However, in the last five years there has been an increased amount of field research and I am confident that someone will find a means of increasing these fungi so they can be available for extensive use in commercial agriculture. If the next 10 years of mycorrhizal research are as productive as the past 10 years, you can expect this to occur.

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PRACTICAL APPLICATION OF ECTOMYCORRHIZA RESEARCH

TRIALS AND TRIBULATIONS OF AN ECTOMYCORRHIZAL FUNGUS INOCULATION PROGRAM

By

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Introduction

When the organizer's of this Conference asked me to talk on this subject, I quickly agreed because the assignment appeared to be so easy. I could simply discuss the problems we have encountered at the Institute over the past few years in our practical application program. It was only when I stopped to think about these problems that I realized how many of them we had encountered, and how poorly my experience as a research scientist had prepared me for dealing with the problems.

As a research scientist, I learned that successful discoveries in science are a product of good training and knowledge, dedicated interest, hard work, insight, adequate support, and lots of luck. I normally dealt with small numbers of people whose actions relative to my studies were rather easy to control.

A large program for application of science is a whole different ballgame. You still need the ingredients that go into a successful research program, but you also need powerful understanding of industrial bureaucracy, human nature, multiple interests, profit, cost-benefit analysis, salesmanship, and the gaps in scientific knowledge of your cooperators.

Purpose of Program

The purpose of our practical program with Pisolithus tinctorius was two-fold: (1) To determine the biological feasibility of producing a commercial inoculum in large quantities that was effective in forming abundant ectomycorrhizae on important tree species in both container and bare-root seedling nurseries. (2) To determine the potential value of the fungus in reforestation and reclamation programs throughout the Nation. Our purpose and motivation are to have the ectomycorrhizal technology applied on an operational basis to improve forestry practices.

What of the motivation of our industrial cooperators? It is important to realize that the basic motivation of industry is profit -- preferably in the short term. Unfortunately, profits from mycorrhizal technology applied to seedlings come primarily when those seedlings become mature trees and are harvested. Forestry is a long-term venture with very few quick returns on investments.

Numerous Cooperators

We have been working cooperatively to develop inocula of Pisolithus tinctorius and other ectomycorrhizal fungi with three companies: Abbott Laboratories and Sylvan Spawn Labs for production of vegetative inoculum and International Forest Seed Company for production of various spore inocula. Their primary purpose is to produce inoculum in commercial quantities that is comparable in effectiveness to the vegetative inoculum we produce in small quantities for research purposes. In all of this work, our research vegetative inoculum served as the control.

Since 1976, we have undertaken over 180 discrete container and bare-root tree seedling tests in 37 different nurseries in 27 states and Canada. Some 16 tree species are involved. Where nursery tests have been successful (Pt indices of 50 or more), with either commercial formulations or our inocula, the seedlings have usually been outplanted. Currently, we are maintaining over 75 outplanting tests in over 20 states with 12 species of forest trees.

As you can imagine, these tests require excellent long-term cooperation, not only with the producers of commercial inoculum, but also with pulp and paper companies and state forestry commissions, federal forestry groups, and various others.

In a majority of cases we have obtained excellent cooperation, but in a few cases conflicts have arisen. Many problems experienced by our cooperators were caused by us. We could not compromise the biological demands of the fungi to accommodate the goals of industry. Many problems we experienced were caused by cooperators wanting conclusions and recommendations faster than the results of studies permitted.

Pertinent Questions

I would like to believe that our trials and tribulations have been instructive -- that they will be helpful to other people who may want to conduct programs similar to ours in the future. In planning such a program, there are 11 questions that I believe must be addressed. The answers, in large measure, will determine the size and shape of the program.

1) How much is known about the benefits of the ectomycorrhizal technology you hope to introduce on a practical basis? The answers to this questions are vital to your cooperators. They are much more likely to invest time, money, manpower, and equipment if the benefits have been fairly well established.

2) What are the potential problems in moving from a laboratory to a commercial scale? After several years, we are still trying to overcome difficulties encountered in mass production of commercial vegetative inoculum.

3) Do your cooperators understand how much they must rely upon you to oversee the production procedures? Our commercial inoculum producers made certain unfortunate changes in production procedures without conferring with us. These changes reduced effectiveness of inoculum that was used in nursery tests. Thus, the actions of one cooperator affected the success achieved by another cooperator.

4) Do you have adequate procedures for testing and can you be assured of quality control? In our work it was necessary to develop procedures and criteria for determining the effectiveness of inoculum. We had to learn to distinguish specific target ectomycorrhizae from native ones and to quantitatively assess their relative concentrations.

5) Do you have sufficient knowledge of the operations and problems of the potential users of your technology? Transfer of mycorrhizal technology requires a thorough knowledge of nursery management and reforestation procedures and problems.

6) Are you prepared to deal with variations in procedure from different commercial operations? No two tree nurseries in the United States are operated the same or have identical soils and weather and use the same cultural practices. Literally dozens of different pesticides, fertility regimes, and the like are in use. The variations can influence results of inoculation tests. It has been necessary for us to gamble that certain variations would be insignificant, because there was no practical way to research all the possible interactions in any reasonable amount of time.

7) Can you maintain control over the quality of the product? We had to convince industrial cooperators that it would be unwise to distribute unproven inoculum to anyone who wanted to test it . . . even though they did not know how. One negative test with prototype inoculum by these people can reverse the momentum you have established from dozens of positive tests.

8) Do the cooperators understand your role in the research and development program? A successful program requires that all information be available to anyone who is interested. Cooperators must understand that all information generated from cooperative studies will be available to you and to all cooperators for contemplation and eventual publication. It cannot be regarded as proprietary information.

9) Do your cooperators understand the potential problems and the time that may be required to succeed? Growth chamber studies and pot studies do not mimic the real world. In controlled environments, fungi and seedlings are not exposed to the same stresses that are encountered in reforestation sites. Tests of inoculum effectiveness require three to seven months in container nurseries and one to three years in bare-root nurseries. After successful nursery tests, at least two additional years are needed to evaluate the effects of inoculation on reforestation or reclamation sites.

10) Have everyone's responsibilities been defined and communicated? Our program is very complex and requires close control over the actions of many people. The size of the management problem should not be underestimated. A tractor driver who has not been properly instructed can apply a pesticide or fertilizer improperly and ruin an entire experiment without anyone being the wiser. Once a nursery study is installed, the nurseryman becomes the most important person in the research program.

11) Are the economics of implementation understood by all parties? Mycorrhizal inoculation requires additional investments in tree nurseries, but these investments must be carried through to tree maturity and harvest. Producers of commercial inoculum must understand that forest managers will resist major increases in reforestation costs. Many users will want to make cost-benefit analyses before routinely applying mycorrhizal technology on an operational scale. They need to know how much better artificially inoculated seedlings are likely to perform in the field compared to seedlings they are now planting. Studies shorter than three to five years will not be sufficient for such analyses. In the meantime, inoculum producers want to market inoculum as soon as possible because they have made large investments in it's development.

When we were starting our practical application program at the Institute seven years ago, we were charting an unknown course. Not only did we not know the answers to some of the questions I have listed, we did not even know what questions to ask. If you are starting a similar program, I hope that the questions provided will save you some time, money, and frustration, and help you to avoid many of the problems we encountered.

PRODUCTION OF ECTOMYCORRHIZAL FUNGUS INOCULUM BY SYLVAN SPAWN LABORATORY

by
Stephen B. Maul

There are two questions that I am often asked. The first is: What is spawn? In addition to fish eggs, spawn is defined by Webster as "...the seed, germ or source of something" and "mycelium... for propagating mushrooms."

The second question is: What is Sylvan Spawn Laboratory? Sylvan Spawn Laboratory originated as a department of Butler County Mushroom Farm, Inc. Butler County Mushroom Farm, or BCMF, is the largest mushroom farm in the world, producing about one million pounds of mushrooms a week. Mushrooms are produced on compost. We make about 700 tons of compost each day. The compost, after pasteurization, must be inoculated with mushroom spawn, a pure culture of mushroom mycelium.

Most microbial products are produced by liquid fermentation. This means that the nutrients are dissolved or suspended in a liquid phase. To provide oxygen for growth, these cultures are stirred constantly. This violent mixing may damage the delicate hypha of fungi causing unnatural growth or even lack of growth. In contrast, mushroom spawn is produced by a solid state fermentation process. The term "solid state fermentation" refers to growth of micro-organisms on solid materials without the presence of free liquid. While the presence of moisture is necessary in a solid state fermentation, it exists in an absorbed or bound state within the solid matrix. Mushroom spawn is grown in this manner on a moist, sterile solid nutrient which remains undisturbed during growth. This provides a much more natural environment for mycelial growth.

BCMF has been producing mushroom spawn on sterilized rye grain for more than 40 years and currently uses more than two million pounds of this pure culture inoculum each year. Until recently, all of this inoculum was produced by a very laborous process using gallon jugs. The jugs were individually filled with rye grain, calcium carbonate and water; sterilized in a large autoclave; individually inoculated; and then shaken on a modified paint shaker to mix the inoculum through the sterile grain. The jugs were shaken again a week later to redistribute inoculum, then shaken a third time after it was fully grown to remove it from the jugs for use as spawn.

About three years ago, we developed and patented a new process for spawn production. Sylvan Spawn Laboratory, Inc. came into existence as a separate entity at that time. The heart of the process is a large rotating vessel used for sterilizing the substrate. It is a fully jacketed pressure vessel allowing the material inside to be steam sterilized under pressure. After cooling, the inoculum is mixed with the substrate before being dispensed into sterile bags in a laminar flow clean room. The bags have a specially designed breather strip allowing ventilation, while still maintaining sterile conditions within the bag. Heat sealed bags are then stacked on carts for incubation in environmentally controlled growing rooms. Figure 1 shows a bag of fully grown mushroom spawn.

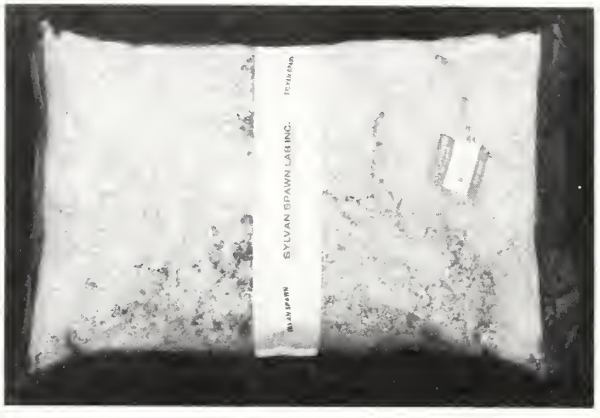

Figure 1. Bag of Mushroom Spawn.

Pisolithus tinctorius Inoculum

Various forms of ectomycorrhizal fungal inoculum have been used to inoculate seedlings; however, pure mycelial or vegetative inoculum has been repeatedly recommended as the most biologically sound method of inoculation (1, 2, 3, 4).

Procedures for producing vegetative inoculum of ectomycorrhizal fungi for research purposes have been developed by several investigators. The method of Marx and Bryan (5) is probably the most commonly used method for laboratory production. This procedure uses a modified Melin-Norkrans liquid nutrient (MMN) absorbed on vermiculite and peat. Except for the substrate, this procedure is very similar to our old method for producing mushroom spawn. With only slight modifications to our new mushroom spawn making process, we are able to produce effective mycorrhizal inoculum. Marx and Cordell (6) have tested our Pisolithus tinctorius inoculum produced on the MMN nutrient and found it to be equivalent to their own preparations.

One of the problems with the MMN nutrient is that after growth the preparation must be leached and dried. This increases the cost and may introduce contaminating microorganisms. In addition, some delicate fungi may not survive the leaching and drying steps. We think we have improved the process by developing a new nutrient that does not require leaching to remove excess nutrients. After several rounds of modifying and testing this nutrient, we have a product that does not require washing or drying, and is effective at inoculating trees (6).

It is also important to minimize the cost to the users. Marx and coworkers found in bare root nurseries that by banding the inoculum under the seed, the amount of dried inoculum needed could be reduced by two-thirds. They developed an applicator for this purpose. However, this

applicator would not work with our moist inoculum. We developed and patented a new applicator that works well with wet or dry inoculum. This applicator is being produced and sold by Whitfield Forestry Equipment, Inc. The applicator applies a two inch band of inoculum at an adjustable depth. Shoes first open up a two inch wide furrow. A band of inoculum is metered into the furrow. The furrow is then closed. The seed is applied directly above the inoculum by a conventional seeder.

We visualize our product going directly to the grower as a pure culture, still sealed in and protected by its growing container. Using the applicator, the unleached inoculum and seed can be applied in a single operation requiring no more time than a normal seeding operation.

Other Species

We at Sylvan have also been producing inoculum of other ectomycorrhizal fungi on both a contract basis and for research. Some of the species that we have grown are:

- Laccaria laccata
- Hebeloma crustuliniforme
- Cenococcum graniforme
- Lactarius rubrilacteus
- Scleroderma aurantium
- Rhizopogon ellenae
- Rhizopogon occidentalis
- Rhizopogon vinicolor
- Rhizopogon vulgarsis
- Suillus brevipes
- Suillus granulatus
- Suillus luteus
- Suillus tomentosus
- Boletus edulus

This is not an exhaustive list and, in many cases, we have more than one isolate of these species. Many of our isolates came from Dr. Trappe and coworkers.

Drs. Trappe and Molina and coworkers, as well as several other investigators, have evaluated our Laccaria laccata and Hebeloma crustuliniforme inocula. They are effective at greater dilution rates than our Pisolithus tinctorius inoculum and have a shelf life of at least six months under refrigeration. We have prepared inoculum of the other species of ectomycorrhizal fungi. These are good, viable cultures; but little is known yet about their effectiveness at inoculating trees.

Different types of planting sites may require different combinations of host and mycorrhizal fungus. Once these combinations are identified, Sylvan will be able to make inoculum appropriate for the specific situations.

Research Needs

1. What is the most effective and economical means of inoculating seedlings with vegetative inoculum? With some species, like Pisolithus tinctorius, Laccaria laccata and Hebeloma crustuliniforme, simply mixing vegetative inoculum with the planting medium is sufficient. Other inoculations seem to be more difficult. If some of these more difficult ectomycorrhizal combinations are particularly useful, can special techniques be developed to improve inoculating success? These techniques might be implemented in containerized nurseries.

2. How can we predict good host-fungus-site combinations?

3. When are the benefits of inoculation worth the added expense?

4. Once some of the above are answered, how can the technology be transferred from the research stage to commercial growers? In the cases of Hebeloma and Laccaria, this may not be a large problem, but what about more difficult inoculations?

We at Sylvan are specialists in growing fungi. We cannot do a large amount of testing to answer the above questions. On the other hand, many investigators interested in testing mycorrhizal inoculum have difficulty in doing so because of a lack of equipment or experience in inoculum production. Our approach has been to cooperate with experts in forestry and mycorrhizae at various universities and experiment stations by producing inoculum that those investigators are interested in testing. In this way, our expertise complement each other. If you have a strain that looks promising and are ready to do larger scale testing, we would like to assist you. Or, if you would like to test a strain that we already have in our culture collection, that can also be arranged.

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MICROBIAL INOCULANTS FOR TISSUE-CULTURED ECTOMYCORRHIZAL AND ACTINORHIZAL TREES

By

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Keywords--Alnus incana, Frankia spp., Hebeloma crustuliniforme, in vitro propagation, Pisolithus tinctorius, rooting

Introduction

Propagation by tissue-culture allows for the production of genetically uniform crops from selected plant materials. The process can yield superior tree lines without prolonged conventional breeding, thus providing a powerful tool for improving tree performance and forest productivity (Bonga and Durzan, 1982).

Tissue-culture systems have been developed for many forest tree species, most of which associate with mycorrhizal fungi, and some of which are colonized by nitrogen-fixing bacteria as well. Because many of these trees benefit from inoculation with these symbiotic microorganisms, it may be desirable to routinely inoculate tissue-cultured tree crops during nursery production. In theory, tissue-cultured trees may benefit more from such inoculations than do seedlings. First, micropropagated trees are often multiplied as shoots which are then rooted on mist benches. Rooting of microshoots can be very difficult. Mycorrhizal fungi can enhance the rooting of woody plant cuttings (e.g. Lindermann and Call, 1977; Navratil and Rochon, 1981), and may have similar effects on micropropagated shoots. After rooting, tissue-cultured trees are often transplanted to containers for nursery production. Containerized plant production systems are particularly amenable to microbial inoculation (Trappe, 1977; Tinus and McDonald, 1979), in part because synthetic soil mixes may contain little indigenous inoculum.

Methods for tissue-culture propagation and microbial inoculation of woody plants have improved markedly in recent years, but there have been few attempts to inoculate tissue-cultured trees. This paper describes our initial efforts to inoculate micropropagated Alnus incana (thin-leafed alder) with ectomycorrhizal fungi and the nitrogen-fixing actinomycete Frankia. The objectives were to evaluate the effects of inoculation on rooting, survival, and greenhouse growth of the trees, and to determine the optimal stage in the transplant-production process for inoculation.

Materials and Methods

A. incana tissue-cultures were initiated from seedling shoot tips and propagated on Lloyd and McCown's (1981) low-salt woody plant medium enriched with 20 g l^{-1} sucrose, 1 micromolar benzyladenine, and 5 g l^{-1} agar. The cultures were maintained at 22±1° C under 16 hours day^{-1} of fluorescent illumination. Microshoots were selected for the experiments from shoot clumps formed after two three-week culture cycles.

Ectomycorrhizal fungal inoculum of Hebeloma crustuliniforme (S-260) and Pisolithus tinctorius (S-431) was produced in vermiculite-peat substrate saturated with modified Melin-Norkrans medium as per Marx and Kenney (1982). Two-liter jars of inoculum were incubated for eight weeks at 22±2° C in the dark. Harvested inoculum was rinsed with tap water for ten minutes prior to use.

Frankia isolate NPI 01310609 was isolated from A. rubra root nodules collected in Tillamook County, Oregon, in October of 1982. Cultures were grown in a propionate-yeast extract medium (Benson, 1982) at 28±1° C for three weeks. Culture aliquots were homogenized, centrifuged to determine packed-cell volume, and resuspended in sterile distilled water.

The microshoots were rooted in soil-less mix composed of quartz sand, perlite, and vermiculite (1:1:1;v:v). The rooting experiment was a two-way factorial combination of treatments in a randomized complete block design. There were three fungal treatments; Pisolithus, Hebeloma, and an uninoculated control. There were two actinomycete treatments; Frankia and an uninoculated control. Each treatment combination had 20 shoots in each of three replicate flats. Fungal inoculum was substituted for one half of the vermiculite in appropriate treatments. Frankia treated flats received liquid cell-suspension at the rate of 73 microliters (packed-cell volume) per flat. The plants were maintained at 95% relative humidity, 22±1° C temperatures, and 3.7×10^{5} lumens cm^{-2} fluorescent illumination. They were fertilized weekly with 20% strength Hoaglands solution without phosphate (Hoagland and Arnon, 1950). After three weeks, the plants were removed and the rooting response of each shoot was rated on a scale of one to five, depending on the presence and length of primary and secondary roots. The ratings were as follows: a one rating indicated no rooting; two indicated one to three primary roots without laterals; three meant three or more primary roots without laterals; four indicated three to five primary roots, some of which had lateral roots longer than 3 mm; a rating of five meant there were five or more primary roots with laterals longer than three mm. The shoots were independently rated by three researchers who had no knowledge of the applied treatments. The root systems were then examined for mycorrhizae and Frankia nodules. Data regarding the rooting index and the percentage of shoots which rooted in each flat were subjected to two-way analysis of variance. Significant differences between treatment means were identified by Tukey's W Tests (Steele and Torrie, 1980).

The plants were then transplanted into 165 ml Ray Leach Conetainers® containing Turface® (arcillite clay) and vermiculite (1:1;v:v). At this time, a third treatment (reinoculation) was imposed upon the experiment, resulting in a three-way factorial arrangement in completely randomized design. One half of each previous treatment group was transplanted without reinoculation, while the other half was reinoculated at the time of transplant with the same combination of microbial inoculants it had received during rooting. Thus, any particular combination of inoculum treatments was applied either at one or both of two stages in the production process. Fungal inoculum was again substituted for one half of the vermiculite in appropri-

ate treatments. Frankia inoculum was applied as a liquid cell-suspension at the rate of 0.83 microliters (packed-cell volume) per plant. The plants were maintained in a greenhouse at 25±15° C, with 60% full sunlight at midday. They were fertilized five days per week with 20% Hoaglands solution without nitrates or phosphates, in order to avoid nutritional inhibition of nodulation or mycorrhiza formation. Turface® contains sufficient phosphate for normal alder growth, but symbiotic fixation was the sole source of nitrogen. After ten weeks (November to February), the plants were harvested and evaluated for survival, mycorrhiza formation, nodulation, total plant dry weight, and shoot height. Survival and growth data were subjected to three-way analysis of variance. Significant differences between treatment means were identified by Tukey's W Tests.

Results and Discussion

Fungal inoculation strongly affected rooting of A. incana microshoots (Table 1). Comparing treatment groups which did not receive Frankia inoculum, flats which received Hebeloma inoculum had significantly higher percentages of shoots which rooted. In those same groups, inoculation with either fungus significantly improved overall rooting as measured by our index. There was extensive Hebeloma hyphal proliferation within developing root systems, but neither fungus formed mycorrhizas during the rooting period. This rooting enhancement may be attributable to fungal production of plant-growth stimulating substances (Slankis, 1973), but could also have been due to favorable effects of the inoculum on rooting mix chemistry (e.g. lowering of pH caused by fungal metabolic products). A determination of the mechanisms involved and the reliability of such rooting enhancements will require further work. Frankia did not significantly affect rooting response (Table 1). In those treatments which received Frankia, there was no significant fungal enhancement of rooting.

The survival of A. incana plants following transplant to the greenhouse was also influenced by inoculation with ectomycorrhizal fungi (Table 2). Treatment groups which received either of the fungal inoculants during rooting had higher rates of survival than did non-inoculated control groups. This could have been due to improved rooting or improved root function resulting in greater stress tolerance among the inoculated plants. Frankia inoculation had no discernible effect on transplant survival (Table 2).

Inoculation of tissue-cultured A. incana with ectomycorrhizal fungi did not significantly affect plant growth in the greenhouse (Table 2), even though one of the fungi vigorously colonized root systems. Hebeloma crustuliniforme formed typical alder mycorrhizas with dense hyphal sheaths. Hartig nets were limited to the outer cortical layers. No mycorrhizas were observed on plants treated with Pisolithus inoculum. Plant growth within treatments was highly variable. Our general experience indicates that there is substantial inherent variability in the growth rates of tissue-cultured plantlets of some alder genotypes. More effort must be directed toward the careful selection of uniform tissue-cultured alders in order to minimize this variability. It cannot be concluded from these results that ectomycorrhizal inoculation affected A. incana growth in the greenhouse. However, previous experience (Loree, unpublished data) indicates that H. crustuliniforme can form mycorrhizas and enhance the growth of both A. incana and A. rubra seedlings under similar conditions. Tissue-cultured plants should respond similarly once they are acclimatized to greenhouse conditions.

Table 1. Effects of inoculation with Pisolithus, Hebeloma, and Frankia on rooting of tissue-cultured Alnus incana microshoots. Means within a column not sharing a common letter differ significantly (P=0.05).

Inoculum treatment	Mean rooting index (1-5 scale)	Mean % of shoots rooted
Uninoculated control	3.2 a	83 a
Pisolithus	3.9 b	97 ab
Hebeloma	3.8 b	100 b
Frankia	3.4 ab	93 ab
Frankia + Pisolithus	3.4 ab	92 ab
Frankia + Hebeloma	3.7 ab	92 ab

Frankia inoculation resulted in marked improvements in plant growth (Table 2). Frankia, either alone or combined with Hebeloma, caused significant increases in mean shoot height and/or mean plant dry weight when applied during both phases of plant production. If applied only during rooting, Frankia enhanced plant growth significantly only if it was applied in combination with Pisolithus inoculum. Pisolithus may have allowed for earlier Frankia colonization, or otherwise improved the ability of Frankia to persist through transplantation.

The stage of plant production in which inoculum is applied is important. Hebeloma and Frankia applied during rooting initiated colonization, which persisted through transplant and proliferated in root systems during greenhouse growth (Table 2). However, reinoculation at transplant significantly increased the percentage of plants within treated groups which were ultimately colonized by either organism (Table 2). It seems that the optimal stage for inoculation, and the relative benefit of reinoculation, may depend on the intent of the grower and his choice of inoculants. If microbes are chosen to enhance root development, inoculation on the rooting bench will be most effective. If reliable establishment of long-term symbiosis is more important, reinoculation during greenhouse growth may be desirable.

These results exemplify the problems and opportunities of research involving symbiotic associations of tissue-cultured woody plants. Great difficulties remain in the in-vitro propagation of many tree

Table 2. Effects of inoculation and stage of inoculation with Pisolithus, Hebeloma, and Frankia on survival, growth, and microbial colonization of tissue-cultured Alnus incana. Treatment means within a column not sharing a common letter differ significantly (P=0.05).

Inoculum treatment	Time of application	Mean plant survival (%)	Mean shoot height (mm)	Mean plant dry weight (mg)	Mean % of plants nodulated	Mean % of plants mycorrhizal
Uninoculated control	None	80 ab	24 ab	15 a	0 a	0 a
Pisolithus	Rooting	100 c	24 ab	15 a	10 ab	0 a
	Rooting and greenhouse	100 c	25 ab	15 a	7 a	0 a
Hebeloma	Rooting	100c	25 ab	15 a	0 a	47 b
	Rooting and greenhouse	93 bc	21 a	15 a	0 a	80 c
Frankia	Rooting	80 ab	40 bc	162 abc	42 c	0 a
	Rooting and greenhouse	73 a	49 c	212 bc	70 de	0 a
Frankia + Pisolithus	Rooting	93 bc	49 c	277 c	63 d	0 a
	Rooting and greenhouse	90 ab	39 bc	131 abc	87 e	0 a
Frankia + Hebeloma	Rooting	100 c	39 bc	133 abc	27 bc	80 c
	Rooting and greenhouse	97 bc	43 c	136 abc	87 e	77 c

species. In particular, rooting of micropropagated shoots presents great challenges. This is especially true of conifers, many of which are highly dependent on ectomycorrhizal fungi. Obviously, fungal inoculation of those conifer species which can be rooted in vivo is a promising research area. Further, the transfer of in vitro propagated plants from the mist bench to the greenhouse is a particularly stressful period. If inoculation with beneficial soil microorganisms can minimize plant loss during this stage, inoculation costs could easily be offset. Finally, whether greenhouse growth is enhanced or not, it is most important to be able to reliably inoculate tissue-cultured trees during nursery production so that symbiosis is well established before field outplanting.

Acknowledgments

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THE APPLICATION OF PISOLITHUS TINCTORIUS ECTOMYCORRHIZAE IN FOREST LAND MANAGEMENT

By

Charles E. Cordell

Keywords--Technology transfer, forest tree nurseries, field forestation, mineland reclamation, alternative inoculation techniques

Although mycorrhizal associations between tree roots and fungi are the rule in nature, the fungus species that form mycorrhizae on a particular tree species in a particular nursery may not be the best one for ensuring good survival and growth of the tree seedling after outplanting. We believe that a particular ectomycorrhizal fungus, Pisolithus tinctorius (Pt), for example, tailors seedlings of some conifer species for planting on adverse sites such as areas disturbed by mining, kaolin spoils, and borrow pits (Marx, 1980; Wolf, Cordell, and Keller, 1982). What is true for seedlings produced in nursery beds is even more true of those produced in the highly artificial environment of containers. In containers, artificial inoculation may be the only practical way to establish ectomycorrhizal associations (Ruehle and Marx, 1977).

Extensive research under controlled conditions and field studies by many cooperating forestry agencies in bare-root and container nurseries have repeatedly demonstrated the value of Pt inoculations. Such treatments have increased the numbers of plantable seedlings produced in nursery beds (reduced culls), and it has increased tree survival and growth after outplanting on a variety of sites. Field studies during the past 12 years have included over 100 bare-root nursery tests in 38 states (Marx and others, 1984), 18 container nursery tests in 9 states (including Hawaii) (Marx and others, 1982), and over 100 field outplantings in 13 states.

During the past 5 years, the Pt mycorrhizal program has evolved from primarily research to a mixture of research, field trials, and practical demonstrations (Molina, 1977; Cordell and others, 1981). The technology has advanced from a research scale to a practical scale (Cordell and Webb, 1980). We believe it is time to get this technology applied on a routine basis in forest tree nurseries. The time has arrived for what the Forest Service calls technology transfer in the field of Pt mycorrhizal manipulation.

This paper describes the methodology for Pt inoculation and the problems involved in a Pt ectomycorrhizal technology transfer program.

Mycorrhizae Technology Transfer

A program to transfer mycorrhizal technology has recently been developed by the USDA Forest Service. Our goal is to put this valuable biological "tool" to work in forest tree nurseries, field forestation, and mineland reclamation. Mycorrhizae technology transfer or research application is a "selling job." Some of the more important components of this program will be discussed in detail.

Benefits

Use of Pt inoculum in bare-root and container seedling nurseries has repeatedly provided significant increases in seedling fresh weights and ectomycorrhizae along with decreased plantable seedling losses (25% - nursery cull reduction). Some outplantings of several pine species artificially inoculated with Pt in the nursery have shown as much as 25 percent better survival and growth than the same uninoculated species with naturally occurring ectomycorrhizae after 10 years in the field. In addition, several mineland reclamation outplantings established with selected pine species in the central U.S. and inoculated with Pt mycelium inoculum in the nursery have shown 20 to 30 percent survival increases as compared with the same uninoculated species after 1 year in the field. Artificial Pt mycelium nursery inoculations have also produced 20 percent increases in the field survival of longleaf pine which is a difficult species to reforest in the Southern United States. Encapsulation of seeds with spores, spore pellets, and spores applied in hydromulch to nursery seedbeds have provided satisfactory Pt ectomycorrhizae in certain nurseries and under certain conditions. However, spore inoculum generally has not been as effective in Pt ectomycorrhizal formation as mycelial inoculum.

Costs

The estimated costs of three primary types of commercial Pt inoculum are summarized in Table 1.

Table 1. Commercial Pt inoculum costs.[1/]

Pt inoculum type	Cost/1,000 seedlings	Cost/planted acre (hectare)
Mycelium	$10.00	$7.26 ($17.94)
Spore encapsulated seeds	$ 2.22	$1.61 ($3.98)
Spore pellets	$ 1.65	$1.20 ($2.97)

1/ Estimated commercial Pt inoculum costs vary with such factors as inoculum costs, application rate, seedling density, seed size, and field planting spacing. Above cost estimates are for Southern United States loblolly and slash pine bare-root nurseries and forestation plantings (1.8 x 3.0 m or 6 x 10 ft. spacing; 1,794 trees/ha or 726 trees/a).

Commercial Inoculum Sources

During 1983-84, no Pt or other ectomycorrhizal fungus mycelial inoculum was available on a commercial basis. Abbott Laboratories postponed its commercial production of Pt MycoRhiz® following the 1982 season. The future availability of commercial Pt inoculum from Abbott Laboratories is questionable, primarily because of recurrent inoculum production problems and adverse economic conditions. The USDA Forest Service is presently exploring alternate sources of commercial Pt inoculum. In 1983 and 1984, Pt mycelial inoculum produced by Sylvan Spawn Laboratories, Worthington, Pa. was tested in several nurseries in the Southern and Central United States. Preliminary bare-root nursery inoculation results with some formulations of this inoculum are very encouraging and suggest that a Pt mycelial inoculum source may be commercially available in the near future. Alternative Pt inoculum sources include spore-encapsulated seeds, spore pellets, and bulk spores--all commercially available from International Tree Seed Co., Birmingham, Ala. Results obtained from several nursery tests of spore-encapsulated seeds and spore pellets during the past 4 years demonstrate that these types of inoculum have good potential for use in certain bare-root and container nurseries.

Application Alternatives

The mycelial inoculum applicator (Fig. 1-a) has been consistently effective for bare-root seedlings of 6 to 8 pine species in over 10 nurseries in the Southern and Central United States during the past 5 years (Cordell and others, 1981). The machine has been modified and improved and is presently produced and marketed by R. A. Whitfield Manufacturing Co., Mableton (Atlanta), Ga.

Several formulations of spore inoculum afford attractive alternatives to the mycelial inoculum. Spore-encapsulated seeds can be sown with conventional seeders (Fig. 1-b), and spore pellets and bulk spores can be applied to the nursery seedbed after seeding with a modified fertilizer applicator (Fig. 1-c) or a standard hydromulch machine (Fig. 1-d). Each of the above application techniques has its advantages and disadvantages for operational use in container and bare-root nurseries. The selection of a particular technique will be based on such factors as effectiveness, cost (inoculum and equipment), seedling use (forestation, reclamation, Christmas trees), and nursery conditions.

Expertise - Technical Assistance

Expertise in a variety of disciplines is required to provide technical assistance in mycorrhizal technology. A broad understanding of nursery, forestation, and mineland reclamation management practices is needed for the successful accomplishment of a mycorrhiae technology transfer program. Particular emphasis should be given to the practical and sociological aspects of the program (Cordell and Webb, 1980). Although these two components significantly affect the success of the technology transfer program, they are frequently either omitted or de-emphasized in the planning phase. Without the successful incorporation of these two major components into the technology transfer program, however, the ultimate success of the program will be severely limited.

Limitations and Problems

As might be expected, a variety of limiting factors and problems are frequently encountered in a mycorrhizae technology transfer program. One of the most important in the nursery application of the Pt ectomycorrhizal research technology is the lack of effective commercial Pt mycelial inoculum. This single factor has postponed the operational use of Pt in both container and bare-root nurseries during the past 3 to 4 years. As previously stated, however, present nursery study results with an alternate source of Pt mycelial inoculum suggest the availability of an effective commercial inoculum within the next year.

In both bare-root and container nurseries, soil and container medium pH, fertility (particularly phosphorus and nitrogen), and soluble salt levels have repeatedly affected the success of Pt ectomycorrhizal inoculations. Certain systemic fungicides used to control specific nursery disease problems (Bayleton® - southern fusiform rust) have recently been found to have significant negative effects on both artificially-inoculated Pt and naturally-occurring ectomycorrhizae development. The fungicide Bayleton could be a highly significant limiting factor in the future operational application of ectomycorrhizal fungi such as Pt in Southern United States bare-root nurseries. The widespread adoption of mechanical seedling harvesting operations in United States nurseries during the past 10 years has significantly affected harvested seedling quality through reductions in feeder-roots and ectomycorrhizae retention. In general, the more recently developed "full bed" harvesters have been superior to the "row bed" harvesters in the retention of both feeder-roots and ectomycorrhizae on harvested seedlings.

Future Applications

During the past 5 years, a number of federal, state, and private agencies in the Southern and Central United States have expressed considerable interest in using Pt ectomycorrhizae in the field forestation of selected species on specific sites. Interest in the use of Pt ectomycorrhizae for mineland reclamation in the Central United States is particularly high. The Ohio State Division of Mineland Reclamation has recently formulated plans for the operational use of Pt ectomycorrhizal seedlings in future reclamation work. Consequently, the potential operational use of Pt ectomycorrhizae in selected forestry and mineland reclamation programs appears highly favorable in the near future. The successful technology transfer and

ultimate utilization of this unique biological "tool" will be primarily determined by the factors summarized in the following formula:

$$TT_{Pt} = \frac{E \times I \times A \times P^2}{C}$$

TT = Technology transfer
Pt = P. tinctorius - ectomycorrhizal fungus
E = Expertise - technical assistance
I = Effective commercial inoculum
A_2 = Effective application alternatives
P^2 = Perseverance and patience
C = Recurrent biological, environmental, cultural, economical, practical, and sociological limiting factors

Discussion and Conclusion

Forest tree nursery and field planting results are very encouraging for the effective, practical, operational use of Pt ectomycorrhizae for custom seedling production. The present technology transfer program will emphasize the use of Pt ectomycorrhizae in selected forestation and mineland reclamation programs. The necessity for improvements in seedling harvesting, packing, storage, shipping, and field planting operations will also be emphasized to the nurserymen and field planting crews. Nursery and field forestry personnel must become acquainted with the "two living organism" (seedling + fungus) concept. They must know that both organisms are needed, and that the beneficial ectomycorrhizal fungus may frequently be more susceptible to adverse conditions than the seedling. The techniques and procedures obtained from this pioneering program should also be applicable to additional ectomycorrhizal fungi and expanded uses.

The need for quality, tailored nursery seedlings for successful field forestation and mineland reclamation by federal, state, industry, and private forest land managers is becoming increasingly apparent. Although seedling costs represent a minor portion of forestation and mineland reclamation expense, seedling quality is perhaps the most significant factor in successful forestation and reclamation. Consequently, a cost-benefit analysis of producing Pt ectomycorrhizal seedlings for selected forestation and reclamation sites may be favorable in many cases. The recent International emphasis on forestation to meet anticipated wood product demands also places added attention on nursery seedling quality, as well as quantity.

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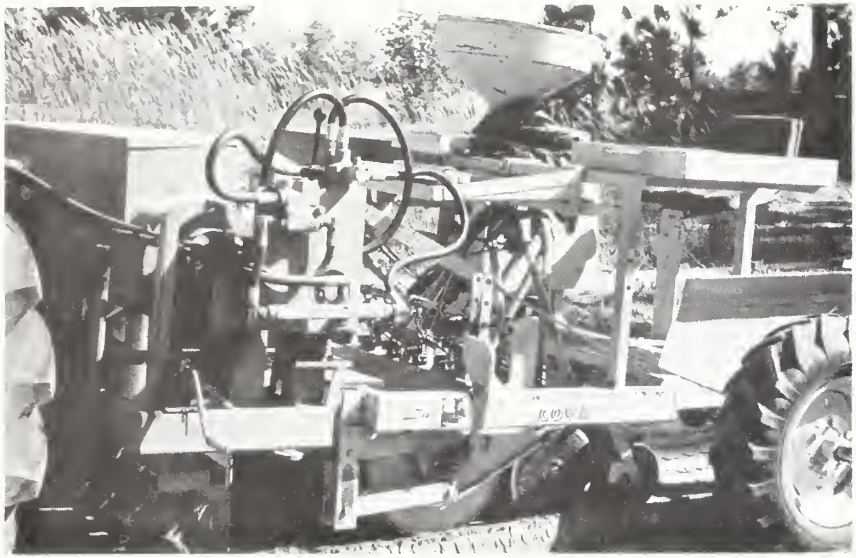

A

B

C

D

Figure 1. Alternative Pt inoculation techniques for bare-root nurseries - (a) mycelial inoculum applicator-nursery seeder, (b) spore-encapsulated seed, (c) spore pellets applied with a modified fertilizer applicator, and (d) spores applied with a hydromulch machine.

PACIFIC NORTHWEST FOREST NURSERY MYCORRHIZAE RESEARCH: BOON OR BOONDOGGLE?

By

P. Morgan

Keywords: Pseudotsuga menziesii, Rhizopogon, Pisolithus tinctorius, ectomycorrhizal and endomycorrhizal inoculation.

Pacific Northwest bareroot nurserymen know that mycorrhizae are vital for normal Douglas-fir (Pseudotsuga menziesii [Mirs] Franco) growth. A north Willamette valley nursery near Canby, Oregon in the mid-1960's dramatized the importance of having mycorrhizae (7). The seedbeds were agricultural soils that were leveled and fumigated prior to sowing. Much of the Douglas-fir crop failed to become mycorrhizal with an average nonmycorrhizal seedling being about an inch tall after two growing seasons. Many seedlings died during the winter between growing seasons; while survivors displayed a prominent purplish cast typical of a severe phosphorus deficiency.

When seedlings do not readily become mycorrhizal the results are startling. Fortunately, most PNW nurserymen have not experienced such a calamity. Instead, local nurserymen tend to take mycorrhizal culture for granted. Somehow, between preparing the soil for fumigation and harvest the seedlings become mycorrhizal. In fact, it would be a difficult task to exclude mycorrhizal fungi and to prevent mycorrhizal formation without drastic measures. Therefore, why go to all the bother and expense to artificially inoculate seed or transplant beds?

Mycorrhizae researchers from the south reported significantly improved increases in seedling survival and growth rates by artificial mycorrhizae inoculation. Pisolithus tinctorius was treated as a fungus with great potential to improve nursery stock and outplanting results throughout the United States. A report by Cordell and Marx (3) indicated that cull rates could be decreased by about 26 percent, while outplanting survival and growth was increased over 25 percent.

Expectations by Pacific Northwest nurserymen were high for P. tinctorius. Studies were installed with enthusiasm in the late 1970's throughout the region. Suddenly, mycorrhizae was an important issue. Local foresters eagerly awaited the promise of planting "p.t. seedlings" on droughty northwest sites. Some nurseries tried to inoculate with spores (1), while many took part in the National Pisolithus tinctorius Ectomycorrhizal nursery evaluation. (2)

Some small successes were noted but the overall attempt by northwest bareroot nurserymen to inoculate Douglas-fir artificially with P. tinctorius did not succeed. Its failure reflected poorly on the mycorrhizal research effort in the northwest. Some nurserymen and foresters were disenchanted with artificial inoculation and considered mycorrhizal research a boondoggle. Ultimately, however the P. tinctorius inoculation program may be a boon to Pacific Northwest forest nursery mycorrhizae research. The excitement generated, although not yet fulfilled, has fostered a cadre of dedicated mycorrhizae supporters. Interest has turned from southern isolates of P. tinctorius to native species of ectomycorrhizal and V.A. mycorrhizal fungi.

Studies of the affect of specific local mycorrhizae on outplanting success are barely underway. Results of field trials to date have not shown a decisive advantage for seedlings artificially inoculated with Laccaria laccata, (Scop. ex Fr.) Hebeloma crustuliniforme (Bull. ex St. Am.) or Rhizopogon species over the standard mixture of Thelephora spp. and L. laccata. Outplanting research may require several years to demonstrate a significant advantage of artificial inoculation of certain native species and strains.

Rather than wait for the need to be discovered in the forest, nurserymen are finding reasons to become mushroom farmers at home. Artificial inoculation of native species can pay benefits before the seedlings are harvested. Natural ectomycorrhizal inoculation, although common, can be unpredictable, erratic and slow. Species that act as pioneer colonizers of northwest nurseries, e.g. Thelephora spp. seem to be well adjusted to nursery culture but not to forest sites. Natural recolonization of fumigated sites by vesicular-arbuscular mycorrhizae is extremely slow and spotty.

Successful artificial ectomycorrhizal inoculation of Douglas-fir in northwest nurseries have included: forest litter from nearby old-growth Douglas-fir sites, spore suspensions of Rhizopogon vinicolor (A.H.Smith) mycorrhizal short roots of Douglas-fir, and commercially produced vegetative inoculation of Laccaria laccata and Hebeloma crustuliniforme Western red cedar (Thuja plicata D. Don) and incense cedar (Calocedrus decurrens (Torr.) Florin) have also been successfully inoculated with V.A. mycorrhizae via root cuttings from mature western red cedar trees.

Perhaps the most aggressive native ectomycorrhizal group are the Rhizopogon spp. This diverse genus is commonly found as a mycorrhizal associate of Douglas-fir in Pacific Northwest forests. (8). Pilz (5) noted that Rhizopogon spp. was one of the most abundent mycorrhizal associates of newly planted Douglas-fir seedlings. Mycorrhizal trials at the D.L. Phipps State Forest Nursery near Elkton, Oregon have found that Rhizopogon spp. are well adapted to bareroot nursery culture, are readily introduced via forest litter, and can be maintained through barefallow periods and survive fumigation. Laccaria laccata and Hebeloma crustuliniforme are more rapid root colonizers than Rhizopogon spp.; however, the latter tends to dominate a root system while the former species generally share 30 to 60 percent with Thelephora terrestris (Ehrl. ex Fr.)

The inoculation techniques are unsophisticated in the northwest but the results are promising. Ten-week-old Douglas-fir inoculated with commercially produced vegetative inoculation of L. laccata averaged 17 percent taller than nonmycorrhizal controls (unpublished data, 4). About 24 percent of uninoculated western red cedar seedlings were less than 2.5 cm tall and only about 4 percent were greater than 10 cm tall after one growing season; while inoculated seedlings had only about 16 percent under 2.5 cm and had about 12 percent more than 10 cm. (unpublished data, 4). These results indicate that artificial inoculation could help alleviate a commonly observed problem of erratic first-year growth of northwest bareroot seedlings. Having more uniformly vigorous seedlings at the end of the first growing season means a better start for the second season, less chance of winter damage or susceptibility to disease, and more harvestable 1+0 seedlings for transplanting or outplanting.

Another benefit of artificial inoculation could include greater protection from first-year seedling root diseases. Sinclair (6) noted that L. laccata inoculated Douglas-fir seedlings had less root lesions, yielded fewer isolates of Fusarium oxysporum (Schecht. emend. Snyd. & Hans.) and had greater heights and weights than uninoculated contrasts. Artificial inoculation of L. laccata may reduce first-year mortality and growth losses due to Fusarium spp. because seedlings could become mycorrhizal prior to the midsummer Fusarium root rot outbreaks. This would allow the use of less seed and fungicides.

Mycorrhizal research at Pacific Northwest forest nurseries is probably a decade behind the vigorous southern program. The screening out of readily adapted species and the start of an outplanting evaluation program is underway. More research is needed to develop methods of large scale inoculation techniques suitable for northwest nursery culture. Also, further studies are needed to inoculate a wider array of species of seedlings and monitor subtle affects of inoculation programs. As nursery research advances, more custom mycorrhizal seedlings will be available for large scale plantation evaluation throughout the northwest. The most exciting times for mycorrhizae work in northwest reforestation may be just ahead.

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PRACTICAL APPLICATION OF VESICULAR-ARBUSCULAR MYCORRHIZA RESEARCH

ASSESSING THE POTENTIAL FOR WIDESCALE VA MYCORRHIZAL INOCULATION

L.K. Abbott and A.D. Robson

Keywords--phosphorus, infectivity and effectiveness of indigenous VA mycorrhizal fungi, Mediterranean environment

Introduction

The potential for widescale VA mycorrhizal inoculation will vary from one situation to another. Our research has been directed towards assessing the role of VA mycorrhizal fungi in phosphate uptake and growth of annual pasture plants in a Mediterranean environment. Some aspects of the philosophy we have developed is equally applicable to other situations whereas other aspects are not.

In a programme aimed at assessing the potential for widescale VA mycorrhizal inoculation, it is first necessary to define the conditions where inoculation increases plant growth. Alleviating a deficiency of phosphorus is the most likely reason for inoculation (see Abbott and Robson, 1982a). VA mycorrhizas may also alleviate deficiencies of zinc and copper (e.g. Gilmore, 1971), but in our acid soils the cost of inoculation would probably be greater than application of zinc and copper fertilizers. Inoculation may be feasible on other soils where zinc and copper fertilizers may lose their availability to plants more rapidly. Inoculation with mycorrhizal fungi may also be worthwhile if it improves soil structure by stabilizing aggregates (Tisdall and Oades, 1979).

In our studies we have focussed most attention on phosphate because in our low-input extensive agriculture the rate of fertilizer application giving maximum profit is less than that required for maximum plant growth. Commonly, phosphorus sufficient to give only 60% of maximum plant growth is applied. Therefore there is scope for increasing plant growth by increasing the abundance of mycorrhizal fungi effective at increasing phosphate uptake. To achieve any level of growth below the maximum, approximately half as much phosphate is required for the growth of mycorrhizal plants as is required for that of non-mycorrhizal plants (see Abbott and Robson, 1984). Losses of phosphorus by leaching, erosion and 'fixation' are directly related to the amount of fertilizer applied and hence reduced fertilizer application that should follow from successful establishment of inoculant fungi will also decrease these losses.

The conditions necessary for inoculation to increase plant growth at any particular site are:-

(i) phosphorus is limiting for plant growth in this soil;
(ii) a suitable inoculant fungus is available for this soil;
(iii) there is a low abundance of indigenous VA mycorrhizal fungi, or those present are not capable of increasing plant growth to the maximum possible given the phosphorus available to the plant in this soil.

Points (i) and (iii) involve identification of sites where inoculation should be successful. A suitable inoculant fungus (ii) must be selected to match the conditions defined in (i) and (iii).

Assessment of adequacy of phosphorus for plant growth

How do you assess the adequacy of phosphorus for plant growth? One way is to use soil and plant analysis. To do this it is essential that these empirical tests have been calibrated against plant response for the plants and soils to which the analyses are to be applied. These tests can identify sites where fertilizer application will increase plant growth (Ojala et al., 1983). However, infection by indigenous mycorrhizal fungi can change the relationship between plant response and soil test (Stribley et al., 1981). A surer but more time-consuming way to assess the phosphorus status of a soil for plant growth, especially if indigenous mycorrhizal fungi are present, is to establish complete responses of plant growth to added phosphorus. Using this method it is clear that the actual amount of phosphorus added to achieve maximum yield has little absolute meaning.

It is possible that plant growth may not respond to applied phosphorus even when the soil analysis gives a value below the critical level. This could occur if another nutrient or factor was limiting plant growth. In such a case, it is unlikely that host growth will respond to inoculation unless the effect of this other nutrient or factor is overcome first.

Assessment of the abundance of indigenous VA mycorrhizal fungi

How do you assess the abundance of the indigenous VA mycorrhizal fungi? What measure of abundance is best related to the effectiveness of that population in increasing plant growth? In many studies, the most effective fungi are those that infect rapidly and extensively.

Spores can be counted and their viability measured but this gives little indication of infectivity of particular fungi. This is because some fungi may form few spores relative to the amount of root they infect, or they may form small spores that are difficult to count. Furthermore, spores are not the only propagules. The most probable number method more accurately estimates the number of viable propagules, but again it gives no indication of the expected rate and extent of infection that may be formed. This is probably related to large differences among fungi in either the size of the propagules or the extent of their aggregation. We believe that a relative measure of the amount and rate of infection formed by the propagules present, i.e. infectivity, is of greater value as a measure of abundance than is an estimate of the number of propagules. For these reasons we have estimated the potential infectivity of fungi from field soils by growing plants and measuring the rate and extent of infection formed 2, 3 and 6 weeks from sowing using subterranean clover as the standard host. The soils we tested fell into three categories:- (1) where infection was rapid and extensive, (2) where

infection had a lag but reached a high level, and (3) where infection remained relatively low (Fig. 1, Abbott and Robson, 1982b).

The morphology of infection formed by some species of VA mycorrhizal fungi within roots is characteristic (Abbott, 1982). We have thus been able to quantify the development of infection by different species of fungi in roots of plants grown in field soils containing mixed populations of VA mycorrhizal fungi. In our agricultural soils, the propagules of different species of fungi that are present in soil at the end of summer, prior to seed germination, differ markedly in the rate at which they initiate infection (Abbott and Robson, 1982b). For example, Acaulospora laevis is consistently slow in its rate of formation of mycorrhizas from our field soils, even when large numbers of its spores are present. By contrast, infection by Glomus species is much more rapid.

Assessment of effectiveness of the indigenous fungi

The next question is - how effective are the indigenous fungi at enhancing plant growth? Differences in effectiveness that are not related to infectivity can only be assessed by establishing the fungi in pot culture, choosing equally infective inocula and comparing fungi at the same level of infection (including the same rate of development of infection). The difficulty in meeting these requirements is indeed very great.

In field soils, differences among fungi in the rate at which they form mycorrhizas with particular hosts may be much more important for their ultimate contribution to plant growth than is their effectiveness measured with 'equal' inocula (as described above). Hence, while A. laevis has been shown to be very effective in increasing plant growth when 10-week old root inoculum is used (Abbott and Robson, 1981), this fungus only infects slowly from the propagules present in our field soils prior to the commencement of plant growth (Abbott and Robson, 1982b). Perhaps when assessing the likely effectiveness of indigenous VA mycorrhizal fungi for increasing plant growth in field soils, we should use those propagules that are present in the soil, prior to the commencement of plant growth, as inoculum.

Potential for inoculation

We now have a knowledge of the phosphate status of the soil for growth of the host we plan to use; we know how infective the propagules in the soil are and we know whether or not the species present are effective or not at increasing plant growth. What is the potential for a beneficial effect of increasing the abundance or changing the population of VA mycorrhizal fungi in this soil (Table 1)?

If there is a high abundance of indigenous VA mycorrhizal fungi that are effective at increasing plant growth, there is a low potential for a beneficial effect of increasing the abundance of the fungi in this soil.

If the fungi are present abundantly but they are ineffective at increasing phosphorus uptake and plant growth, the potential for increasing plant growth by replacing these fungi with more effective fungi is high. However, is it possible to replace the indigenous VA mycorrhizal fungi with inoculant fungi? Our experience in introducing VA mycorrhizal fungi into soils containing highly infective populations of VA mycorrhizal fungi has not been promising (Table 2; Abbott and Robson, 1984b). Our assessment at this stage is that it will be relatively difficult to displace the indigenous population of VA mycorrhizal fungi if they infect rapidly and extensively. If the background population of VA mycorrhizal fungi is low, the potential for increasing the background population appears to be high irrespective of whether the fungi are effective or ineffective at increasing plant growth. However, it is important to determine the cause of the low abundance in case there is an underlying reason that would also limit successful establishment of the inoculant fungi. The low level of infectivity may be due to several factors - e.g. soil erosion, growth of a non-mycorrhizal crop, fallowing, herbicide application, fumigation or soil amendments such as lime. If the low abundance is related to soil erosion, non-mycorrhizal hosts, fallowing or fumigation, there may be no detrimental effect of these factors on the successful introduction of inoculant fungi. Herbicides may persist in soil and limit the success of inoculation. If liming an acid soil to ameliorate aluminium toxicity reduces the population of indigenous VA mycorrhizal fungi, it may also permit the successful introduction of inoculant fungi adapted to alkaline soils.

Providing the factors associated with low abundance are not likely to affect the successful establishment of inoculant fungi, then inoculation is potentially possible whether or not the indigenous population is effective or ineffective. If the indigenous fungi are effective, it may be possible, as an alternative to inoculation, to change the management in such a way as to encourage a greater amount of root to become infected by these fungi. For example, on soils where the accumulation of organic matter is increasing soil acidity, lime application may increase mycorrhizal infection by species adapted to the original, more alkaline soils. Another possibility may be to sow a crop which is usually heavily infected by mycorrhizal fungi; its growth should increase the abundance of mycorrhizal fungi available for initiating infection of subsequent crops.

If the potential for inoculation is shown to be high by the criteria outlined above, the next problem is to see if inoculant fungi are available in a suitable form and quantity which are likely to succeed as inoculants in the particular soil. Procedures for the selection of inoculant fungi are discussed elsewhere in these proceedings (see also Abbott and Robson, 1982a; Menge, 1983). It is essential to choose inoculant fungi that are matched to a particular soil/environment/cropping system to maximize the chance for successful establishment.

It is also essential to check to see whether inoculant fungi have been established successfully, even where clear growth responses have been recorded. In some soils, the characteristic morphology of infection within roots can be used to determine whether inoculant fungi have been successfully

introduced (Table 2; Abbott et al., 1983). Moreover, detailed observations on the development of infection by both indigenous and inoculant fungi are valuable in determining whether enhanced plant growth was indeed associated with increased infection or was merely an artifact of inoculation. To date the fluorescent antibody technique has not been used to assess the success of inoculation of VA mycorrhizal fungi into field soils.

We are confident that the technical problems related to inoculum production will be overcome. But right now there should be greater emphasis on the selection of inoculant fungi and the prediction of sites and situations where inoculation has maximum chance of success. The economic assessment of the value of inoculation will depend upon the cost of inoculation (inoculum production and introduction) relative to the value of savings in fertilizer required to achieve the same yield or the value of increased production if fertilizer use is not reduced concurrently with inoculation.

Table 1. Is inoculation necessary (assuming P is deficient for plant growth)?

(a) If soil has high infectivity of indigenous VA mycorrhizal fungi.		
Effectiveness	+	-
Potential for inoculation	low	high
Strategy	none	inoculation
(b) If soil has low infectivity of indigenous VA mycorrhizal fungi.		
Effectiveness	+	-
Potential for inoculation	high	high
Strategy	inoculation or management	inoculation

Table 2. Success of introduction of G. fasciculatum into four field sites (from Abbott et al., 1983).

Soil	% Root Length Infected		
	Total in uninoculated plants		G. fasciculatum (above background level)
	~4 weeks	~7 weeks	~7 weeks
1	20	50	3(1)*
2	36	77	3(18)
3	15	68	6(0)
4	8	43	27(0)

* G. fasciculatum in uninoculated control in brackets.

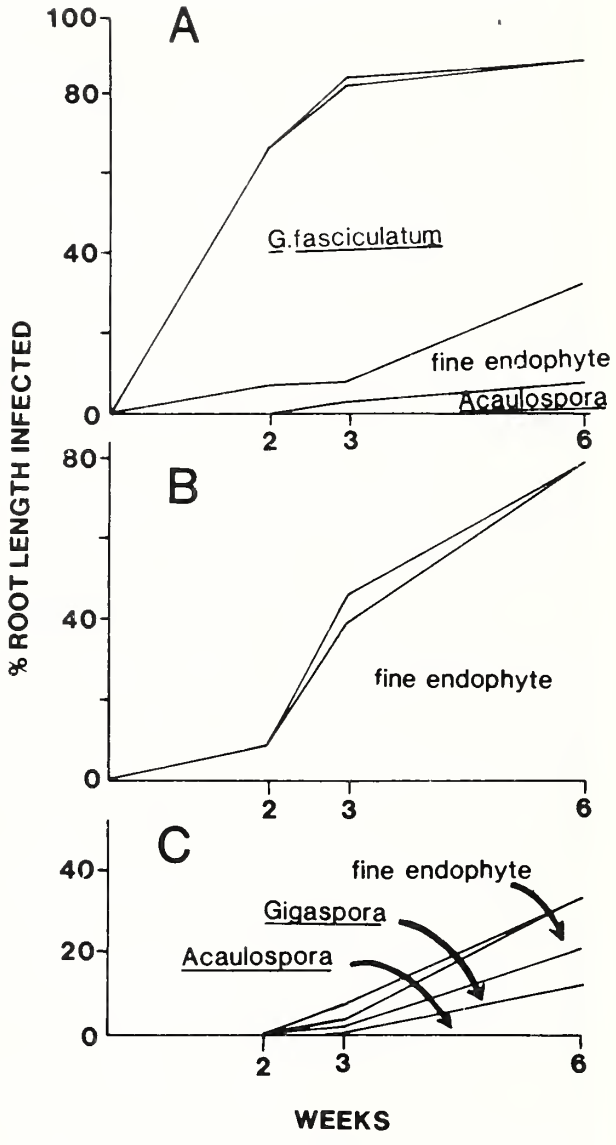

Figure 1. Infection of roots of subterranean clover by indigenous VA mycorrhizal fungi from three (A-C) untreated field soils at 2, 3 and 6 weeks after sowing (from Abbott and Robson 1982b).

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DEVELOPING WIDESCALE VA MYCORRHIZAL INOCULATIONS: IS IT PRACTICAL OR NECESSARY?

By

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Keywords--Inoculation, efficiency, commercialization, indigenous VA fungi, inoculum, carbon drain

Should we develop widescale vesicular-arbuscular mycorrhizal (VAM) inoculations? At the present time the answer is no! Given our current knowledge such widespread inoculations are neither practical nor necessary. There are agricultural situations where VAM inoculations are commercially feasible and highly desireable (Abbott and Robson, 1982; Menge, 1983). Potentially there are many more commercial agricultural systems which could benefit from VAM inoculation. We must search for these specific areas, identify them, and document the beneficial effects of VAM fungi. In order to maintain scientific credibility of both VAM symbionts and researchers on VAM fungi we must recommend mycorrhizal fungi for use only where research proves success and not where there appears to be a high potential for success. Greenhouse, laboratory, or small plot tests have not proven adequate to indicate areas for commercial application of VAM fungi. Large-scale tests under agricultural conditions are required to prove the benefits of VAM in commercial situations. For the most part, such trials have either not been done or have provided negative results which have not been widely circulated. Therefore, the main reason why widespread inoculations with VAM fungi are not warranted is--we have not yet proven VAM fungi to be widely beneficial under commercially acceptable conditions.

There are many reasons why we cannot now nor may we ever be able to recommend widespread VAM inoculations. I will attempt to briefly document some of these reasons.

Mineral nutrition of soils - Many soils are so naturally fertile or receive fertilizers to an extent that mineral nutrients, particularly phosphorus, are present in amounts which provide concentrations in plant tissue which are above the critical concentrations necessary for optimal growth. Under these conditions VAM fungi can provide few benefits for the host and will not increase crop growth (Menge, 1983; Pairunan et al., 1980). These conditions are most often achieved in greenhouse and nursery soils but they frequently occur in field soils as well (Menge et al., 1981). Formulae which rely on quantities of certain soil nutrients can be used with reasonable accuracy to identify soils in which VAM fungi can provide growth responses to specific crops when indigenous VAM fungi are absent (Menge et al., 1981). The nutrient status of any soil should be examined before VAM inoculations are recommended. Soils should have P levels which result in tissue concentrations which are less than the critical concentrations necessary for optimum growth, but not so deficient that VAM fungi cannot improve the tissue concentrations to a level near the critical concentrations.

Host mycorrhizal dependency - Mycorrhizal dependence is the degree to which a plant is dependent upon the mycorrhizal conditions to produce its maximum growth or yield at a given level of soil fertility (Gerdemann, 1975). A wide range of mycorrhizal dependencies in crop responses has been observed. Plants like Leptospermum, Liriodendron, Citrus and grape have extremely high mycorrhizal dependencies in which mycorrhizal plants can be 1200 to 13,000% larger than non-mycorrhizal plants. These plants are good candidates for VAM inoculations. Plants such as sugar beets are non-mycorrhizal and, of course, should never be inoculated with VAM fungi. However, there is a whole array of intermediate plants. Some of these, such as wheat, alfalfa, tomatoes, and potatoes, have a rather low mycorrhizal dependency where mycorrhizal plants range from 10-40% larger than non-mycorrhizal plants. Under field conditions growth responses of this magnitude are hard to measure and it will probably never be economically feasible to inoculate these host plants with VAM fungi in the field.

Indigenous VAM fungi - Indigenous VAM fungi are ubiquitous and when present in high populations are capable of producing growth responses which are as good if not superior to most selected VAM inoculants. These fungi can spread rapidly in the field and usually invade fumigated soil within 6 mos (Menge, 1983). Farmers are beginning to understand VAM fungi and consider them a valuable resource. Instead of commercial VAM inoculations, most growers would rather conserve or utilize the indigenous mycorrhizae fungi. Where feasible we must accept these practical and economical practices as viable alternatives to widespread VAM inoculations. The selection of superior VAM strains (Abbott and Robson, 1982) will be a valuable step toward widespread inoculations of VAM fungi. These strains must be able to compete, survive, and spread as well as increase crop growth. Evidence indicates that high VAM inoculum potentials may be as critical as the intrinsic physiology of VAM fungi in increasing crop growth (Abbott and Robson, 1982, 1981a, 1981b, Menge, 1983). Furthermore, selection of superior strains may prove exceedingly complex since the "superior" strain early in the season may or may not be the "superior" strain by harvest, or after fertilization. A carefully selected "cocktail" of VAM fungi which mimics but improves the indigenous VAM flora may prove most satisfactory in the long run.

Until we perfect VAM inoculants and VAM cocktails for non-sterile soils and until we can predict growth responses due to VAM fungi in non-sterile soil, I believe it is fair to recommend VAM inoculations only for sterilized soils or for soils low in indigenous VAM fungi such as fumigated soils, greenhouses, and disturbed sites.

Inoculum quantity - For many perennial crops where VAM infection, but not immediate growth response, is important, the amount of VAM inoculum is not critical since small amounts of inoculum can achieve VAM infection over long periods of time. However, for annual or short season crops, or when indigenous mycorrhizal fungi are competing for infection sites, high VAM inoculum potentials, which result in rapid infection, are necessary to cause a growth response (Abbott and Robson, 1981a, 1981b). When high inoculum potentials are required the amount of inoculum applied under field conditions quickly becomes limiting. Mycorrhizal inoculations, at high inoculum potential, for high density crops like lettuce, onions, or grain crops can involve between 220 and 1700 kg VAM inoculum/acre. Such quantities of inoculum are prohibitive for most commercial farms. Inovative inoculation methods must be designed if widespread mycorrhizal inoculation of these crops is to become a reality.

Inoculum quality - Currently VAM inoculum can only be produced economically under greenhouse conditions in a non-sterile environment. Under these conditions contaminating microorganisms are presently the most serious problem to the production of inoculum. Serious root-rotting pathogens must and can be eliminated using resistant hosts, chemicals, aseptic methods, etc. However, common air and water borne contaminents of greenhouse soil, such as Fusarium, Pythium, Rhizopus, Penicillium, Aspergillus, Papulospora and many others are often impossible to eliminate from greenhouse produced mycorrhizal inoculum. For most host species these inocuous soil fungi would present no health hazard, but if the inoculum is used as widespread field inoculum on many different hosts, it is possible that some of these weak pathogens could damage germinating seedlings or reduce crop growth. It may be necessary to restrict mycorrhizal inoculum for use on specific crops where performance can be guaranteed or a disclaimer statement might have to be issued with inoculum when used on plants for which the inoculum was not designed.

Inoculation methods - For many perennial crops where rapid growth responses are not always necessary, the methods for VAM inoculation are not critical since even poorly placed inoculum will infect over time. However, it is becoming increasingly evident that growth responses in short season crops are achieved with high inoculum potentials and proper placement of inoculum (Abbott and Robson, 1981a, 1981b, 1982; Menge, 1983). Placement of inoculum at some distance directly under the seed is usually the best method for inoculation (Abbott and Robson, 1982; Menge, 1983). However proper timing and placement of VAM inoculum has not been worked out for many crops. Commercial machinery for inoculating crops with VAM inoculum directly under seeds is not available. Commercial fertilizer banding machines which have been used successfully to inoculate crops in the field with VAM fungi are not always dependable since they do not inoculate and seed at the same time and they cannot place inoculum under the seed after they germinate. Widely dispersed or improperly placed VAM inoculum may be wasted, so until carefully conceived inoculation methods are described for more crops, widespread inoculation of crops is not possible.

Cost - Currently the cost for mycorrhizal inoculum is not less than the cost of fertilizer. Most growers will, when given the choice, choose fertilizer since it is familiar and dependable. In order to achieve widespread commercial inoculations we must prove that mycorrhizal inoculations are in someway better than fertilizer applications. This can be done under certain agricultural conditions, but in many others it is very difficult. Unless the cost for mycorrhizal inoculations becomes very low or the cost of fertilizers rises dramatically widespread commercial VAM inoculations are not economically feasible.

Carbon drain - It has been convincingly shown that VAM infections may utilize 6-10% of the total fixed carbon of the host plant (Koch and Johnson, 1984). It seems likely that under conditions of plant stress, in young VAM infections before external hyphae are formed, or with certain VAM isolates, which for one reason or another do not absorb mineral nutrients, VAM fungi could result in crop growth depressions. Such VAM induced growth depressions have been repeatedly observed (Cooper, 1975). While the concept of carbon drain may result in increased caution on the part of commercial users of VAM fungi, it appears to be a normal cost of the beneficial effect and it should not prevent commercial use of VAM fungi. It is unlikely that VAM fungi causing permanent growth depressions would ever be recommended for commercial use. However, if widespread inoculation of many different hosts becomes a reality, the chances would be increased that limited pathogenicity may occur under some specific conditions. Therefore, recommendations for VAM inoculations should only be made for soils, crops, and conditions for which VAM inoculations have been thoroughly tested.

Will widespread VAM inoculations ever be practical and necessary? Yes, I believe they will for many specific agricultural areas after careful and thorough analysis of each specific situation. However, for many other agricultural areas, mycorrhizal inoculations will not prove beneficial, feasible, or economical. The number of commercial applications for VAM fungi will increase tremendously if 1) we can select VAM fungi which reproduce, survive, and compete in a variety of non-sterile soils or 2) fertilizer supplies become limiting.

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TECHNOLOGIES FOR INOCULUM PRODUCTION, STORAGE, AND UTILIZATION OF VA MYCORRHIZAL FUNGI: INTRODUCTION

By

David M. Sylvia

It is now well-known that most plants are colonized by VA mycorrhizal fungi and that mycorrhizal dependency varies widely among plant species. In general, coarse-rooted plants grown in soils of low fertility require the mycorrhizal symbiosis to obtain normal growth. The challenge for future applied research on mycorrhizae is to identify commercial operations where it is both biologically and economically reasonable to inoculate plants with VA mycorrhizal fungi. For these situations there is still a great need to improve current methods for the production, storage, and utilization of VA mycorrhizal inoculum.

The lack of a readily available source of VA mycorrhizal inoculum is a major limitation to the commercial utilization of these fungi. Even though VA mycorrhizal fungi can have limited growth in the absence of a host plant, they have not been grown in pure culture and are considered obligate symbionts. Isolates of VA mycorrhizal fungi are, therefore, increased in association with plant roots in pot cultures. These cultures are actually complex systems comprised of host plants, mycorrhizal fungi, soil microorganisms, and the physical and chemical properties of the soil. The quality of inoculum produced in pot cultures can vary widely due to the many interactions among these variables. Contamination of pot cultures with soilborne root pathogens is of great concern to commercial inoculum production. Tim Woods addressed this problem in his paper on large-scale production of VA mycorrhizal inoculum. Future research on inoculum production should focus on developing systems to grow these fungi rapidly under more controlled conditions, possibly in a soilless medium. The nutrient-film technique, discussed by Anne Warner and Barbara Mosse, is an example of such a system.

An area that has received little attention in the commercial utilization of mycorrhizae is long-term storage of inoculum. Much time and labor is required to produce VA mycorrhizal inoculum, but it unlikely that this material can be utilized immediately on a large-scale. For this reason, methods must be found to preserve inoculum viability during storage. Inez Tommerup has utilized L-drying and cryropreservation to store VA mycorrhizal fungi, and she discussed these techniques in her paper.

Methods for the utilization of VA mycorrhizal fungi will vary with the host plant, cropping system and cash value of the crop. The specific techniques employed will need to be prescribed on a case-by-case basis. In each situation, however, an attempt should be made to utilize efficient strains of VA mycorrhizal fungi. The concept of mycorrhizal efficiency was, therefore, discussed by Lynette Abbott.

Current research on specific methodologies for utilizing VA mycorrhizae concluded this session. John Menge discussed inoculation methods in high-value citrus nurseries, Ray France and Mark Colemen presented data on a low-cost method to increase mycorrhizal propagules in a hardwood tree nursery, and Jim Graham discussed strategies for introducing inoculum into artificial media for container-grown plants.

There was no attempt in this workshop to provide detailed information on standard methods for manipulating VA mycorrhizal fungi. This information is available in the recent book edited by N.C. Schenck (1982). Rather, the purpose of this workshop was to stimulate interest in developing new and improved methods for the production, storage, and utilization of VA mycorrhizal fungi.

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COMMERCIAL POT CULTURE INOCULUM PRODUCTION: QUALITY CONTROL AND OTHER HEADACHES

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Large scale production of VA mycorrhizal inoculum is technically feasible using traditional pot culture techniques developed thirty years ago by Barbara Mosse and Jim Gerdemann. Since that time, advances in our understanding of factors controlling the initiation and spread of VAM colonization have provided at least a theoretical basis for optimizing the production and sporulation of VAM fungi in these dual culture systems. In practice, however, pot culture techniques remain as much an art as a science, and problems exist in regulating inoculum quality. The most serious problems involve maintenance of high inoculum potential and control of contamination by airborne pathogenic fungi and other pests.

Within the next six months, NPI will be producing its first commercial batches of VAM inoculum through pot culture. Our production facility is a 400 m^2 greenhouse with double polyethylene walls, a continuous concrete floor, and expanded metal benches, all of which can be disinfested regularly. Irrigation water is passed through a 5 µm filter to remove most fungal spores, invertebrates, and other contaminants. Photoperiod lighting is used throughout the facility to maintain production during the winter months. And, temperatures are regulated with a four stage system involving natural gas furnaces, evaporative coolers, and exhaust fans. Our facility has the capacity to produce 80,000-100,000 liters of pot culture inoculum annually.

In production trials to date, we have produced inoculum of Glomus deserticolum, G. intraradicies, G. etunicatum, G. mosseae, and Gigaspora margarita on a reliable basis. After 2-4 months of culture (depending upon the season, the host, and the VAM fungus) our inoculum has typically contained 100-120 g (fresh weight) of roots per liter with 50-75% VAM colonization, 100-1,000 VAM fungal spores per milliliter, and no detected contamination by pythiaceous fungi, nematodes, or insects and insect larvae. These pests have been reliably controlled with strict sanitation and the use of pesticides shown to be non-deleterious to VAM fungi. Cross contamination of VAM fungi between cultures and sporadic contamination by airborne fungi such as Fusarium spp. and Rhizoctonia spp. remain problematic. Airborne contamination is most severe during the summer months when large volumes of air are drawn through the house for cooling.

We are relatively pleased with this record, but we anticipate intermittent quality control problems as we move into commercial production. As such, we are concerned with defining acceptable limits for inoculum quality, i.e., with setting standards for those levels of colonized roots and fungal spores which constitute acceptable inoculum potentials, and for specifying those contaminants which, if present, render inoculum unuseable or unsaleable.

At present there are no such guidelines for mycorrhizal inoculants. Three states in the U.S. (Illinois, Ohio, and Wisconsin) as well as Canada regulate the quality of Rhizobium inocula with statutes specifying standards for:

1) labeling (typically the names of the inoculum producer and distributor, the batch number, and the expiration date must be given);
2) inoculum potential (lower limits for the number of live bacterial cells per seed or gram of inoculum and the percent of plants that must become nodulated in standardized trials are specified); and
3) contamination (Canada requires that contaminating microbes must not be at such levels in inoculum as to affect the performance of the Rhizobium).

As mycorrhizal inoculants become commerically available, similar standards are likely to be set for these products by state and federal agencies. We would like to be involved in defining those guidelines, and would welcome comments and suggestions from mycorrhizal scientists and industry representatives concerning their views on standards for VAM inoculum quality.

THE NUTRIENT FILM TECHNIQUE FOR INOCULUM PRODUCTION

By

A. Warner, B. Mosse, and L. Dingemann

Keywords--Vesicular arbuscular mycorrhiza, sphagnum, peat

Introduction

The nutrient film technique (NFT) as described by Cooper (1975) is widely used in horticulture mainly for tomato and cucumber production. The technique is a modified solution culture system in which the plant roots grow in a shallow layer of flowing nutrient solution. The solution is circulated by pump and pH and nutrient levels regularly monitored and adjusted accordingly. In commercial systems plants are grown with optimum nutrient levels depending on the species.

The use of the NFT system for production of vesicular arbuscular (VA) mycorrhizal inoculum was first described by Mosse et al (1980) in which they monitored VA mycorrhizal infection development in a range of plants and nutrient levels. The inoculum produced consisted of a root mat 10-15mm thick with an extensive veil of fungal mycelium and spores forming outside the roots. However infectivity of the inoculum is greatly reduced by air drying making it unsuitable for longterm storage.

An alternative method of growing plants in the NFT system has been found to be more successful for growing VA mycorrhizal inoculum. In it the plants are sown in a block of rooting medium, which is subsequently transferred to the NFT channel. It is such a commercial system which has been adapted for large scale production of VA mycorrhizal inoculum.

Methods and Materials

The following schedule outlining the inoculum production procedure is dependant on seasonal factors. Lettuce growth and therefore inoculum production is faster in summer than winter and so addition of nutrients and date of harvest have to be adjusted accordingly. The rooting medium is prepared by mixing medium cut sphagnum moss peat (Bord na Mona, Irish Peat Development Authority) with three times its weight of water and limed to the appropriate pH (endophyte dependant) using $CaCO_3$. VA mycorrhizal inoculum is added in the form of infected roots, spores and mycelium and the resulting mixture compressed into $4cm^3$ blocks. A pelleted *Lactuca sativa* (lettuce) seed is placed on each block and they are kept in a propagating glasshouse for 2-4 weeks before transfer to the NFT channels. As no nutrients have been added to the rooting medium the plants are small but root infection levels are generally 20-30%. The NFT channels slope at 1:70 and nutrient solution flows at 200ml min^{-1}. The nutrients listed in Table 1 are added and replenished when required. The solution pH is adjusted regularly using 1M H_2SO_4. Blocks are sampled regularly to monitor infection progress and after 8-10 weeks the circulating solution is switched off and the blocks allowed to dry prior to harvest. The whole block consisting of peat, roots, spores and mycelium is then used as inoculum.

The effect of storage on the peat inoculum was tested. Inoculum was stored either wet (50% moisture content) or air-dried in polythene bags for up to 6 months. A comparison was made with root inoculum produced in the same system but without the peat rooting medium. Infectivity of the inoculum was assessed by measuring the percentage infection in lettuce test plants grown in sterile soil for 30 days and inoculated with 1g (wet weight) of inoculum.

Table 1. Nutrient solution (mg l^{-1})

Rock phosphate	65.6
Fe EDTA	35.0
$MgSO_4$ $7H_2O$	43.7
KNO_3	28.0
$CaSO_4$ $2H_2O$	83.3
Trace element solution	0.8ml l^{-1}
Trace elements	g l^{-1}
$ZnSO_4$ $7H_2O$	0.22
$CuSO_4$ $5H_2O$	0.08
$NaMoO_4$ $2H_2O$	0.27
$CoSO_4$ $6H_2O$	0.05
H_3BO_3	2.86
$MnCl_2$ $4H_2O$	0.18

Results and Discussion

A range of host plants including *Zea mays*, *Pisum sativum*, *Trifolium repens*, *Medicago sativa*, *Lycopersicum esculentum*, *Allium cepa* were screened for suitability in this system, however *L. sativa* proved best adapted, producing large quantities of roots which readily became infected and remained within the peat block. Inoculum production was unaffected by lettuce variety although summer and winter varieties were used when appropriate. The endophytes *Glomus mosseae*, *G. fasciculatum*, *G. macrocarpum*, *G. caledonium*, *G. albidum*, *G. clarum*, *Gigaspora margarita*, *Gi. heterogama* and *Acaulospora laevis* were all multiplied successfully in the system.

At harvest, in addition to infection having spread throughout the root system, much external mycelium had formed and ramified throughout the peat particles binding them to the roots.

Each peat block weighs approximately 110g when wet and 15-20g after air-drying. An equivalent volume of soil grown inoculum weighs 140-200g depending on soil type. Owusu-Bennoah and Mosse (1979) calculated that 2500 kg h^{-1} of soil inoculum was necessary for field inoculation. An equivalent volume of peat inoculum would weigh between 198-235 kg which could be a considerable saving in transportation costs. Using the NFT

system described, 10500m^3 of peat inoculum can be produced per hectare of glasshouse.

The results of the inoculum storage test are shown in Table 2. Infectivity of the peat inoculum was significantly greater than that of root inoculum alone. Air drying slightly reduced the infectivity of the peat inoculum but that of the root inoculum was reduced greatly. Prolonged storage reduced infectivity of all inoculum types. Contamination of inoculum during storage by other non-mycorrhizal fungi occurred frequently with the wet inoculum but air drying prior to storage overcame this problem.

The use of the NFT system for growing VA mycorrhizal inoculum demonstrates how an existing highly mechanised system for growing crop plants can be adapted for inoculum production. The system eliminates the need for soil sterilization prior to inoculation and contamination of the inoculum by other VA mycorrhizal fungi has never been observed.

The method of inoculum production in an NFT system has been patented - Patent Nos. 2043688 and 8310972 - by the British Technology Group, 101 Newington Causeway, London SE16BU.

Table 2. Percent infection in lettuce roots (mean 4 reps) inoculated with stored NFT inoculum.

Inoculum	Days Storage				
	0	30	60	90	180
Peat wet	51.9	53.9	47.9	47.5	33.9
Peat air-dried	43.8	39.9	36.1	35.5	28.5
Roots wet	29.9	25.6	21.3	20.9	20.0
Roots air-dried	2.5	1.5	-	-	-
				LSD 10.5	

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STRATEGIES FOR LONG-TERM PRESERVATION OF VA MYCORRHIZAL FUNGI

By I.C. Tommerup

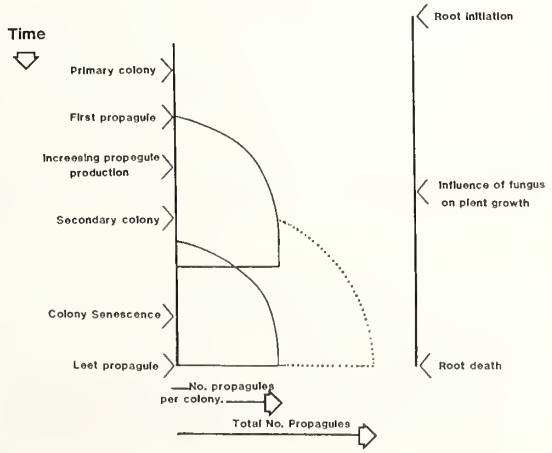
Keywords--Storage, survival, genetics, mutation sub-culturing, Glomus, Acaulospora

The major reason for preserving living cells is to reduce the opportunity for degeneration and mutation in populations. Preservation conserves the diversity in wild populations or the specialised traits in a cloned culture, and avoids the selection pressures inherent in any sub-culturing programme. Fungi have inherent genetic instability and VA mycorrhizal fungi are probably no exception. They have an extensive ability to adapt to different ecological conditions and to exchange genetic information. The capacity for hyphal fusion, an apparent lack of cytoplasmic incompatability between isolates of a species, and a multinucleate mycelium are characteristics which can lead to the rapid exchange or segregation of nuclear or extranuclear genetic information (Tommerup, 1981).

The second reason for developing techniques for the long term preservation of inoculum is as an insurance against contamination or loss and as a means of reducing the cost and time of maintaining cultures.

A third reason for using preservation techniques to store inoculum is to have continuously available supplies of inoculum having defined characteristics, and a consistently high viability and potential to infect plants. While spores can survive for many years in stored, dry soil (Table 1), survival in storage is modified by soil water potential, temperature, and the physiological state of the spores. Spore physiology is determined by the conditions under which they were produced, the time since morphological development was completed, whether or not the spores have a dormant phase, and the environmental conditions since spore development was completed but before spores are put into storage. Hyphae in dried mycorrhizae (Tommerup & Abbott, 1981) in dry soil can survive for at least six years, however, viability can decline by as much as 80%.

TEMPORAL RELATIONSHIPS BETWEEN PROCESSES OF COLONIZATION, PROPAGULE PRODUCTION AND ROOT DEVELOPMENT.



EFFECT ON SPORE SURVIVAL OF VARIATION IN POPULATION AGE & STORAGE CONDITIONS AT 25°C

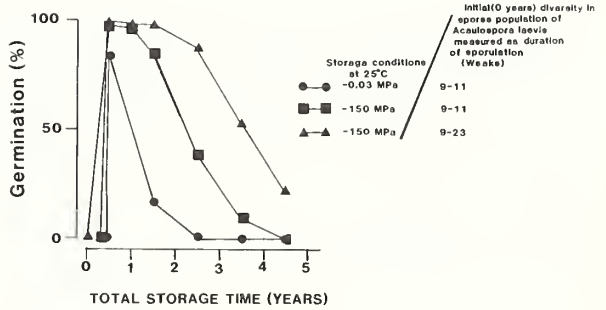
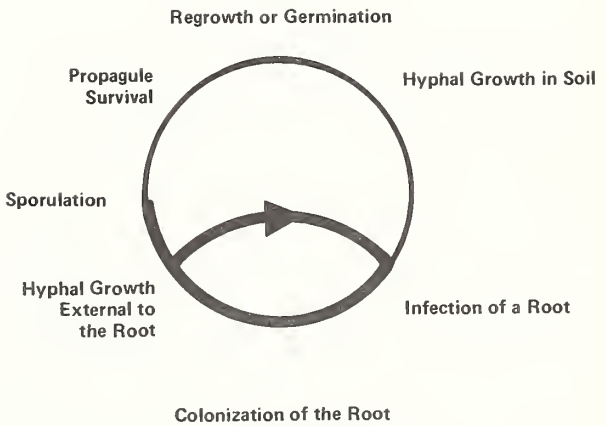


Table 1. Survival of natural inoculum in air-dried soils.

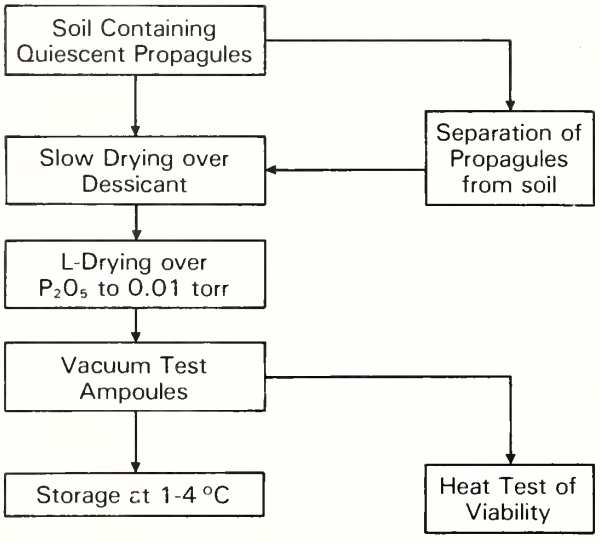
Soil type	Fungus	Storage (years)	Survival % germination
Krasnozem	(Glomus sp.	15	7
Alluvial	(Glomus sp.	18	(4
	(Acaulospora		(13
	(laevis		
Basaltic	(Gigaspora sp.	16	(11
alluvium	(Acaulospora		(22
	(laevis		
	(Glomus sp.		(14
Black earth	Glomus sp.	14	4

By all accounts fungi resembling the VA group have been associated with plants from about the time plants left a watery environment and invaded dry land. The diversity of habitats the fungi occupy and the accompanying diversity in their life histories are only now being documented. Many of the characteristics which enable various developmental phases to survive environmental stress may be usefully exploited as stages for the long-term preservation of the organisms. In some climates spores are a major survival structure, but in other climatic regions the mycorrhizal phase is dominant.



A technique for the preservation of VA mycorrhizal fungi by L-drying has been developed (Tommerup and Kidby, 1979). Hyphae in dry mycorrhizal roots have also been preserved by this method. Preserved

spores and hyphae have 100% survival after six years. L-drying is drying under vacuum to 0.01 torr (1.3×10^{-2} bars) at temperatures above zero. Spores or hyphal root fragments of the following fungi have been preserved by L-drying:- Glomus caledonium, G. monosporum, G. tenue, G. fasciculatum, Gigaspora calospora and Acaulospora laevis. L-drying requires inexpensive, simple equipment that can be constructed in a general workshop. The dried material in sealed ampoules can be stored in a domestic refrigerator.



Some fungi do not readily form spores in natural environments or in pot culture. Preservation of young mycorrhizal segments (spore free), using low temperature techniques, is being developed for fungi with this type of behaviour. For other fungi it will provide a way of storing inoculum without having to wait until cultures produce spores. A wide range of organs, tissues and cell types appear to be genetically stable when stored in liquid nitrogen (-196°C) and this temperature increases stability and survival compared to -136°C or lower (see Ashwood-Smith and Farrant, 1980). A scheme for freezing, storage (long-term survival is currently being tested) and thawing, living mycorrhizae has been developed (Tommerup and Bett, 1984).

Briefly the procedure has the following stages:- (i) Dispersion of segments in non-penetrating cryoprotectant in 1ml in a plastic vial; (ii) freezing by a single step (direct immersion in liquid nitrogen) or by two steps (slow cooling to -40°C and then rapid cooling to -196°C); (iii) thawing rapidly at 40°C and testing the ability of segments to infect hosts.

The capacity of the fungi in mycorrhizal segments to withstand desiccation and freezing indicates that they should successfully freeze-dry and this technique is currently being investigated.

It is important that the viability of populations after preservation by any method is high. The problem with low rates of recovery is that only a component of the population may be surviving. This is especially important where the population is genetically diverse. Low survival rates may also be due to, for example, the physiological state of the cells, or the stage of cell development in sub-populations prior to freezing.

Many variables determine the responses of cells to the stresses of freezing and thawing, or to drying and rehydration. These include intrinsic factors of the fungal cells and extrinsic factors such as cryoprotectants. In a dual system such as hyphae in a mycorrhizal state, there are possible but unknown direct and/or alternatively indirect effects of damage to host cells on the performance of the fungus. The cooling rate and the cryoprotectants modify survival after freezing (Tables 1 and 2; Tommerup and Bett, 1984). Of the procedures tested, dehydration prior to freezing gives the highest survival irrespective of the cooling rate. Now that survival levels following freezing have reached moderate levels, changes in survival of hyphae in segments cooled or thawed at a series of rates can be examined. The possible advantages or disadvantages of other non-penetrating or penetrating cryoprotectants can be compared with the current system.

After L-drying, the highest survival of propagules occurs when the initial rate of dehyration is slow, whether the propagules remain in the soil in which they formed or have been separated from the soil. The inability to survive the initial phase of drying is the main reason for loss of viability in some species such as Gigaspora calospora and Glomus monosporum. During freezing the capacity to survive the initial cooling from 0 to -40°C appears to be the crucial phase in the process. As discussed earlier, combinations of cooling rates and cryoprotectants should lead to improved survival.

Two types of tests have been used to measure the viability of frozen or L-dried cells. One involves testing the capacity of the preserved structures to infect hosts. The other measures the ability of the structures to regrow or germinate when incubated between cellulose or polycarbonate membranes in soil. The differences after thawing or rehydration in the handling procedures for the two viability tests have not affected survival values. Loss of viability therefore appears to have occurred during earlier stages in the process.

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SELECTION OF 'EFFICIENT' VA MYCORRHIZAL FUNGI.

L.K. Abbott and A.D. Robson

Keywords--Infectivity, effectiveness, persistence, inoculum suitability, field soil characteristics

VA mycorrhizal fungi selected to enable more efficient use of phosphate fertilizer must be able to: (i) increase plant growth, (ii) establish and persist at high levels in soil, and (iii) withstand procedures involved in inoculum production and introduction into soils. An inability to meet any of these three criteria disqualifies an isolate from further evaluation.

The major characteristics of VA mycorrhizal fungi important to their potential as inoculant fungi are: their ability to (i) infect rapidly and extensively, (ii) form hyphae in soil which is well distributed for enhancing P uptake, (iii) absorb P from the soil solution, and (iv) form large numbers of propagules which are able to (a) persist in soil, (b) germinate rapidly when required and (c) tolerate procedures involved in large-scale inoculum production and inoculation (e.g. desiccation).

Comparisons of the ability of VA mycorrhizal fungi to increase P uptake and plant growth (i.e. effectiveness) are of limited value unless inocula of equal infectivity are used. If fungi are compared using inocula which differ in the resulting rate and extent of infection, it is not possible to separate the characteristics associated with abilities to take up P from those merely due to differences in the rate and extent of formation of mycorrhizas. Most of the data that demonstrate differences between fungi in their enhancement of plant growth would probably reflect differences in mycorrhiza development if infection development had been studied in greater detail. We are not aware of any data showing that fungi, compared at similar stages and extent of mycorrhiza formation, differ in their ability to absorb P from soil. This is probably because of the difficulty in obtaining similar rates of mycorrhizal development for different isolates of fungi. The type of study needed to show such differences requires many early harvests and an understanding of the effect of inoculum quantity and form on infectivity.

The propagules in inoculum used for comparisons of effectiveness should be those that would be present in field soils at the commencement of plant growth. Hence, in our Mediterranean environment, the propagules should be those that would be present at the beginning of winter. These include spores for most fungi but may also include dried mycorrhizas for some species. Comparisons of fungi for effectiveness also need to be made using a range in propagule number.

Although we have used the term 'efficient' in our title, we do so only to draw attention to the lack of precision in the use of this concept. We prefer to refer to 'suitable or successful inoculant fungi for particular situations' because a fungus may be able to make a major contribution to enhanced P uptake in one set of conditions but not in another. A fungus may be very effective at increasing P uptake but only if it is either present at a high inoculum level or introduced into a particular type of soil. Therefore, to label a fungus as 'efficient' is meaningful only for specified conditions.

Our approach is to select inoculant fungi suitable for a particular site. To do this the two major prerequisites are: (i) an understanding of the biology and ecology of the fungi from among which selection is to be made, and (ii) details of the field site (including host).

The questions that need to be answered about the general biology of the fungi and their ecology are grouped under four headings as follows:

(1) Infectivity - Can the fungus infect rapidly and extensively? What inoculum type or quantity is needed for maximum infection? How much external hyphae is required to maintain extensive infection? Does the fungus have a narrow or wide range in edaphic factors over which it can infect rapidly and extensively? Can the fungus maintain infectivity in the presence of other fungi?

(2) Effectiveness - Can the fungus infect rapidly and extensively? Does the fungus form external hyphae which are distributed in the most suitable way for nutrient uptake? What affects the formation and distribution of external hyphae? How well does the fungus absorb P from the soil solution? Do the hyphae transport and transfer P to the plant faster than it diffuses in soil?

(3) Persistence - Does the fungus form a large number of propagules? Can the fungus survive in soil in the absence of host plant growth? Is the fungus suitable for use in transplanted seedlings?

(4) Inoculum suitability - Does the fungus form a large number of propagules? Is regrowth of propagules rapid and synchronous with the reestablishment of plant growth? Do the propagules survive treatments such as desiccation, L drying and storage? Do propagules maintain viability during and after inoculum preparation and inoculation?

Some of these questions are appropriate for consideration in more than one category. There may be short cuts which can be used to answer some questions. For example, if infectivity and effectiveness are correlated, it may be sufficient just to assess infectivity. Also, if the formation of external hyphae is related to infectivity, the infectivity (which may be easier to assess) could be measured alone.

Using our knowledge of the biology and ecology of the fungi, we can match selected fungi to particular situations if we have also defined the properties of soil: chemical (e.g. pH, P, salinity); physical (e.g. soil structure, infiltration); and microbiological (e.g. species and abundance of indigenous VA mycorrhizal fungi, abundance of any microorganisms detrimental to mycorrhizal formation). Additionally, we have to take account of climatic factors (e.g. rainfall, temperature) which can affect the length of the growing season and choice of host. Management practices (e.g. cultivation, use of chemicals, cropping system) also need to be considered.

With this background, fungi can be chosen which should have a high chance of successful introduction into a particular soil. The type of inoculum and the quantity needed can also be determined using an understanding of the biology of the fungi and the conditions for its planned inoculation.

An example of the type of experimental plan that could be used in selecting suitable inoculant VA mycorrhizal fungi for use at a particular site is presented in Table 1. The emphasis is on the need to match fungi to a particular site.

Table 1. Example of stages in a programme designed to select VA mycorrhizal fungi for field inoculation

Stage 1 -	a. Collection of fungi and definition of soil and climatic factors limiting the distribution of fungi in field soils;
	b. Establishment of pot cultures and studies of the biology of the fungi (e.g. spore dormancy, infectivity of forms of inoculum)
Stage 2 -	Evaluation of infectivity and effectiveness using a wide range of soils
Stage 3 -	Evaluation of suitability of fungi for inoculum production.
Stage 4 -	Evaluation of ability of fungi to establish and persist under field conditions.

We have followed this approach (Abbott and Robson, 1982) in selecting fungi suitable for inoculation into agricultural soils of SW Australia. First, we isolated fungi from local soils to establish pot cultures. Thus the fungi are initially selected from a pool which is climatically suited to our environment. In our case we have a Mediterranean environment - winter rainfall with growing season for annual crop and pasture species and hot dry summers with no crop or pasture growth.

We have tried to shorten the procedure of selecting inoculant fungi effective at increasing plant growth by identifying those factors determining effectiveness. For example, we have generally found that the infectivity of a fungus from a particular source of inoculum is related to the effectiveness of that fungus from that inoculum. Infectivity is not a constant feature of a particular fungus; it may change with the state or form of the inoculum and with inoculum level. Because we have found that infectivity and effectiveness are closely correlated, we consider it is of more value to study factors affecting infectivity than to pursue studies of effectiveness directly at this stage in the selection programme.

However, could we bypass selection based on infectivity by selecting fungi which form extensive external hyphae? That is, is the production of external hyphae correlated with infectivity? In another study we showed that the length of external hyphae formed in soil was not necessarily related to the infectivity of inocula of G. fasciculatum and G. calospora (Table 2). G. calospora formed a greater length of hyphae per cm infected root than did G. fasciculatum. G. fasciculatum increased plant growth but G. calospora did not. Should we therefore be selecting fungi on the basis of the distribution of their hyphae in soil, rather than simply by the quantity or length of hyphae formed? Do we choose fungi with a wide range in tolerance of edaphic conditions (e.g. pH) or do we select fungi with a narrow range in tolerance. Our isolate of G. fasciculatum has a wide range in tolerance and Glomus sp. (WUM 16) has a narrow range in tolerance to soil pH (Table 3). Answers to such questions are essential if we are to reduce the time taken to select fungi for inoculation which are matched to particular sites.

Table 2. Change with time in length (m) of external hyphae formed by G. fasciculatum and G. calospora per cm infected root of subterranean clover.

Inoculant fungus	Harvests (weeks from sowing) 4	5	7
Glomus fasciculatum	14.4±2.5	3.6±1.3	2.4±0.2
Gigaspora calospora	20.8±2.1	31.4±7.0	12.2±2.7

Table 3. Effect of soil pH on the formation of VA mycorrhizas by Glomus fasciculatum and Glomus sp. (WUM 16) in roots of subterranean clover from inocula mixed throughout entire pot.

Soil pH in 1/5 w/v 0.01M $CaCl_2$	Inoculant fungus	% Root length infected <5 cm deep	>5 cm deep
5.3	G. fasciculatum	82a	56bc
	Glomus sp (WUM 16)	0	2d
	Nil	0	0
6.1	G. fasciculatum	90a	66ab
	Glomus sp (WUM 16)	20c	7d
	Nil	0	0
7.0	G. fasciculatum	92a	74a
	Glomus sp (WUM 16)	59b	42c
	Nil	0	0
7.5	G. fasciculatum	86a	74a
	Glomus sp (WUM 16)	59b	47c
	Nil	0	0

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EFFECTIVE TECHNIQUES FOR FIELD INOCULATIONS

By

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Keywords--Inoculation, Inoculum potential

While there are effective methods for field inoculation with vesicular-arbuscular mycorrhizal (VAM) fungi, most of these methods have been recently reviewed (Abbott and Robson, 1982; Menge, 1983; Menge & Timmer, 1982; Powell and Bagyaraj, 1982). The major criticism of field inoculation work is the paucity of large-scale commercial inoculations and the lack of commercial machinery with which to inoculate VAM fungi.

Perhaps rather than to dwell on specific methods in a short discussion, a brief review of the theory and principles of VAM field inoculations would serve to underscore current thinking on the subject. Field inoculations with VAM fungi may be divided into three categories:

1) VAM resupply--To date this is the most common type of VAM inoculation and has provided the most consistent and successful results. The purpose for this type of inoculation, rather than to achieve rapid growth response, is to resupply VAM fungi to soils which have been fumigated or for one reason or another lack VAM fungi. The species of VAM fungi are not critically important. Rapid infection is not necessary. Low inoculum potentials are acceptable since infections will spread with time. Placement of inoculum is not important but is frequently in bands placed beside young plants. Placement may be so late that it often occurs after symptoms of mycorrhizal deficiency have occurred. Such inoculations normally occur with perennial plants such as citrus, avocados, peaches, and grapes which are grown in fumigated soils.

2) Concentrated superior strain--This is currently the main type of inoculation attempted in non-sterile soils or for annual or short season crops. Inoculum potential and placement of inoculum, are very important, since early and rapid infections are desired to get maximum P uptake before plants flower or before competing indigenous mycorrhizal fungi infect. Selected VAM fungi which exhibit characteristics such as rapid infection or enhanced P uptake are preferred.

3) Population restructure cocktail--The primary purpose of this type of inoculum, which consists of mixtures of VAM species selected for specific purposes, is to introduce superior strains into the field environment on a more or less permanent basis. Immediate growth responses occur rarely since the inoculum potential of any given isolate is low. Inoculum placement and timing is not highly critical, since success depends upon superior strains competing and establishing themselves to the long-term benefit of specific crops or specific rotations of crops. Inoculations via seed pelleting must be placed in this category, since inoculum potentials are low and placement is poor. One initial fumigation, which enables the establishment of the selected VAM populations, is highly desirable.

It must be kept in mind that the primary underlying purpose for inoculating with VAM fungi is to achieve optimum P uptake during periods when the P demand by the crop is greatest. Since maximum P uptake is probably directly proportional to the period of maximum viable external VAM hyphae, the growth rates and life cycles of individual species of VAM fungi should be considered when selecting species for inoculation. The time of maximum external hyphae can be further determined by the time of initial VAM infection. The time of initial infection can, in turn, be determined and regulated by the time of inoculation, inoculum potential, placement of inoculum, and the host root growth and distribution. When inoculating crops in the field these factors should be considered in order to achieve the desired effect of increased P uptake at specific times.

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COVER CROPS TO INCREASE INOCULUM IN THE FIELD

By

R. C. France, M. D. Coleman, and M. L. Cline

Keywords--Glomus mosseae, Liquidambar styraciflua, cover crops, hardwoods

Introduction

Hardwood tree species grown commercially in the southern United States exhibit an enhanced growth response to mycorrhizal fungal inoculation. Species, such as Liquidambar styraciflua, Fraxinus pennsylvanica and Platanus occidentalis, form symbiotic associations with VAM fungi. Operational production of hardwood seedlings in forest tree nurseries is expensive, and large-scale inoculation of nursery seedbeds with the appropriate VAM fungi must be cost-effective. Direct inoculation of nursery seedbeds with VAM-infested soil following nursery soil fumigation requires large amounts of inoculum. In addition, care must be taken to minimize seedbed damage during mechanical incorporation of inoculum into nursery soil.

Rotation cover crops are often used in forest tree nurseries to increase the organic matter and inorganic nutrient contents of the soil following a tree seedling crop. In addition, cover crops aid in erosion control and weed control. The nature of the mycorrhizal status of most agricultural plant species used as cover crops offers an opportunity to capture both the classical benefits of cover-cropping as well as the potential for improving the VAM population levels of hardwood nursery soils in situ in a cost-effective manner. From the forestry perspective, the measure of success lies in the production of high quality, both physiologically and morphologically, hardwood seedlings which translates into forest plantations exhibiting high survival and vigorous growth.

Three main topics will be discussed in this paper: (1) summer versus winter cover-cropping for VAM inoculum production; (2) cover crop species selection for improving VAM inoculum levels; and (3) hardwood seedling growth response to the VAM inoculation/cover crop method.

Methods and Materials

Ten summer and five winter cover crop treatments were examined in a microplot nursery study for their ability to increase VAM fungal population levels in the soil and to enhance the growth of the subsequent L. styraciflua crop. Pot cultures of Glomus mosseae were used to inoculate a fumigated silt loam nursery soil at a rate of one part inoculum to three parts soil. Each cover crop was sown at a uniform rate determined by seed weight in three replicated microplots. After germination, cover crops were placed on a nursery cultural management program.

Summer cover crops were sown in June and winter crops in November of the first year. Summer crops were allowed to seed, and total plant biomass was incorporated into the soil in the fall of year 1. Plant matter decomposition occurred for approximately four months. Winter crops were grown for five months with an additional two months for decomposition.

L. styraciflua seeds were sown in March of year 2 in the summer cover crop microplots and in May of year 2 in the winter cover crop microplots. Seeds were sown at such a rate as to produce 10 plantable L. styraciflua seedlings per square foot area in a simulated nursery bed design. After germination, seedlings were culturally managed as in the operational nursery.

Results

I. Cover Crop Phase (Table 1)

After five months of cover crop growth, VAM spore production in the soil, across all summer cover crop species, exceeded that occurring in the soil supporting winter cover crops by four times: 16 versus 4 spores/cm^3 soil, respectively. A comparison in VAM infection levels between summer and winter crops overall showed no difference: 35% in summer and 34% in winter cover crops. Based on VAM infection results, root matter production in the soil must be determined between species before a conclusion as to the significance of this difference can be made. However, it is evident that spore inoculum is produced to a greater extent with the summer cover crops.

Within the summer crops, most species significantly increased the spore density in the soil over the control. In general, the nonleguminous plants ranked higher in spore production than the legumes. VAM infection varied with crop species. Soybean, corn and millet exhibited significantly greater VAM infection than controls. No clearcut relationship between spore production and VAM infection was evident.

Among winter crops, only red clover significantly increased spore production in the soil. However, all four winter cover crop species significantly increased VAM infection. VAM infection with ryegrass was significantly greater than with the other three winter crops.

Table 1. Mean spore density levels in soil and VAM infection levels on summer and winter cover crop species inoculated with Glomus mosseae. Crops grown for five months.

Cover Crop Species	VAM Spore Density (No. spores/cm^3 soil)	VAM Infection[a] (%)
	Summer	
Clover, White	15	27
Corn	17	46
Crowder Pea	14	35
Lespedeza	11	32
Millet	19	38
Milo	16	30
Peanut	12	25
Sorghum/Sudan	23	24
Soybean	13	57
Control[b]	3	24
Tukey's HSD (α= 0.05)	10	11
	Winter	
Clover, Red	8	36
Oats	2	33
Ryegrass	2	52
Wheat	4	15
Control[b]	2	0
Tukey's HSD (α= 0.05)	5	12

[a]Percent of total root segment cortical area infected.

[b]Unplanted controls; VAM infection occurring on volunteer herbaceaous plants in summer cover crop control treatment only.

Table 2. Liquidambar styraciflua seedling growth response following summer and winter cover-cropping. No significant differences were found at α = 0.05 between cover crop species treatments for the four growth characteristics.[a]

Cover Crop Species	Shoot Height (cm)	Root Collar Diameter (mm)	Total Dry Weight (g)	Root/Shoot (g/g)
		Summer		
Clover, White	101.2	15.1	97.5	0.40
Corn	114.3	18.1	139.2	0.63
Crowder Pea	91.7	15.0	81.4	0.52
Lespedeza	103.5	16.1	91.8	0.45
Millet	108.1	17.1	108.3	0.63
Milo	124.2	18.8	148.6	0.58
Peanut	108.7	16.4	101.1	0.57
Sorghum/Sudan	99.0	14.6	82.3	0.54
Soybean	115.0	17.4	139.8	0.48
Control	105.0	14.6	68.7	0.56
		Winter		
Clover, Red	120.5	14.5	63.5	0.40
Oats	101.4	13.9	50.0	0.48
Ryegrass	90.3	11.5	38.1	0.45
Wheat	114.2	13.5	54.7	0.44
Control	81.2	9.7	23.7	0.41

[a]Seedlings grown after summer cover crops were 15 months old at sampling; after winter cover crops--13 months old at sampling.

II. Tree Crop Phase
(Table 2)

Results for L. styraciflua seedling growth after 15 months showed no significant difference between summer cover crop treatments in mean shoot height, root collar diameter and total seedling dry weight. Greatest growth in these three characteristics was attained in the milo treatment, though the soybean and corn treatments also yielded superior L. styraciflua growth. These cover crops also ranked high in VAM spore production and VAM infection levels.

In general, L. styraciflua growth in all nine summer cover crops exceeded seedling growth in the control treatment in root collar diameter and total dry weight. Control seedling shoot height occurred mid-way in treatment ranking. Root/shoot ratios were acceptable in all treatments.

As with the summer crop treatments, seedling growth response after 13 months was not significantly different between winter crops. Significant treatment effects were found in shoot height and root collar diameter at $\alpha = 0.10$. L. styraciflua shoot height, root collar diameter and total dry weight were greatest in the red clover treatment. All four winter cover crop treatments resulted in greater L. styraciflua growth as compared to the control treatment. The lack of volunteer plant species during the winter cover crop phase may be one reason for less growth in control seedlings. Root/shoot ratios were acceptable in all winter cover crop treatments.

Discussion

Summer and winter cover-cropping to increase VAM inoculum in situ is an effective means of inoculating a hardwood nursery crop. Kormanik et al. (1980) reported that corn, millet, sorghum-Sudan and milo were all effective as cover crops for increasing VAM inoculum density in nursery soils. It is less costly than direct pre-sow nursery bed inoculation, requires less VAM inoculum initially on an area basis to attain a comparable tree growth effect and does not require the precision inoculum distribution that nursery bed inoculation does.

The selection of winter versus summer cover-cropping is actually an "and/or" situation which is influenced by "starter" inoculum quantity and production schedule, annual tree crop production demands, the crop rotation cycle of the nursery, and the available plantable acreage of the nursery, among other factors. In general, summer crops increased spore production in the soil to a greater extent than winter crops. Overall, VAM infection levels were comparable between summer and winter cover crops on a root area infection basis.

Typical forest nursery crop rotation cycles in the southern United States include the following: (1) a "1-1" or one-year tree crop and one-year cover crop; (2) a "1-2" or one-year tree crop and two-years cover crop; and (3) a "2-2" or two-years tree crop and two-years cover crop. For a 1-1 crop rotation cycle, only one summer cover crop can be grown. For 1-2 and 2-2 crop rotation cycles, one winter and two summer cover crops can be grown on an alternating basis. However, no winter cover crop can be grown between successive tree crops under a 2-2 crop rotation system due to the time shortage.

Cover crop species selection depends on nursery location, soil type, soil nutritional status and the nurseryman's preference. From a VAM inoculum production standpoint, any cover crop is better than fallowing the land. However, the most cost-effective production scheme dictates the use of the "best" inoculum producer for the particular nursery site. In addition, one should consider the use of leguminous cover crop species for the supplemental nitrogen benefit.

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A MYCORRHIZAL MEDIUM FOR CONTAINER-GROWN PLANTS

By

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Most container crops produced on a large-scale in the greenhouse are grown in soilless media that contain mixtures of peat, bark, vermiculite, and perlite and receive frequent applications of soluble fertilizer. These media are devoid of vesicular-arbuscular mycorrhizal (VAM) fungi. Thus, crops grown in containers for transplant into field soils may benefit from inoculation to increase efficiency of nutrient uptake, plant uniformity, and most importantly transplant performance.

Availability of phosphorus (P) from soluble P fertilizers is greater in soilless media than in soil (1,2). The inhibitory effect of soilless media on VAM colonization and growth response is apparently attributable to the inability of the medium to adsorb P when fertilized with soluble P fertilizer (1, 2, 3). Rock phosphate is a slow-release form of P that allows for VAM colonization and growth responses comparable to that in soil (2, 3). When P-availability is controlled, P-induced micronutrient deficiencies are alleviated by VAM (2, 3). An additional advantage of rock phosphate is long-term availability of P compared to superphosphate which is leached within weeks after application to soilless media (3). Repeated applications of soluble P are required especially for growing woody plants, but no further P fertilization may be necessary if rock phosphate and VAM inoculum are incorporated into the potting mix before planting.

Another factor limiting greenhouse application of VAM fungi is development of forms of inoculum and inoculation techniques applicable to large-scale container production. Although methods for commercial production of inoculum are available, there is still concern about contamination with pathogenic microorganisms. Inoculum should be free of potential soilborne pathogens before it is introduced into the "semi-sterile" media used in container production.

Because VAM fungi only sporulate on the roots of a host plant, the resultant inoculum consists of a bulky mixture of colonized roots in soil (mixed inoculum). Extraction of spores from soil on a large-scale is difficult and storage of spore inoculum for extended periods of time is less reliable than storage of mixed inoculum. Peat-based mixes may serve as a medium to produce a light-weight inoculum that could be more easily transported and stored.

A more practical method for inoculum extraction is to harvest the roots and discard the medium, but most VAM fungi sporulate in the soil around roots and spores are detached when roots are extracted. However, Glomus intraradices Schenck & Smith is unique in that spores are formed in roots. Spores in roots can be efficiently recovered from soil, the roots chopped into fragments and stored up to 9 months at room temperature without loss of colonization potential (Graham, unpublished results).

Ideally, inoculation should occur at the earliest point in production of the plant, e.g., at time of seeding or transfer of tissue culture plantlets, so that maximum benefit from VAM can be realized with the least inoculum. Inoculum is most effective when layered below the seed so that emergent roots will immediately reach the inoculum. This method is not practical for large-scale inoculation of container plants.

The most convenient way to inoculate at time of seeding is to mix the inoculum with the potting medium, but colonization of roots may be reduced by inoculum dilution. When several methods of inoculation with root fragments of G. intraradices are compared, mixing the inoculum with peat-perlite medium is as effective as placing the same amount of inoculum directly under the seed (Graham, unpublished).

In summary, VAM fungi can effectively colonize and function in soilless media if P availability is controlled. Feasibly, root fragments of G. intraradices and rock phosphate could be introduced in a one-step process during media preparation. Furthermore, if moisture conditions in the medium are controlled, it may be possible to store mycorrhizal inoculated soilless mixes for use months later.

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ECOLOGY OF MYCORRHIZAE

MYCORRHIZAL FUNGI: AGENTS OR SYMPTOMS OF TROPICAL COMMUNITY COMPOSITION?

By

David P. Janos

Keywords--competition, niche differentiation, synergism, ectotroph occurrence, carbon cost, specificity, degrees of mycotrophy

Introduction

> "...older phytogeographists may say that...it is the distribution of trees that conditions the distribution of fungi. Such a theory is one-sided and absolutely erroneous, as proved in many silvicultural experiments. ... Neither the fungus nor the tree can develop, and soon succumbs to native species provided with suitable symbionts, in soils lacking adequate fungi."
>
> Tadeusz Dominik, 1951

As suggested by Dominik, failures to establish ectomycorrhizal trees where they are not native and do not form mycorrhizae are well known (Mikola 1973). So too are the limitations imposed by the harsh site factors of mine spoils on colonization by ectomycorrhizal fungi and the hosts that require them (Schramm 1966). Similar failures of mycorrhiza-requiring plants to establish in the absence of vesicular-arbuscular (VA) mycorrhizal fungi have been demonstrated (Janos 1975, Reeves et al. 1979). Few studies have examined the effects of VA mycorrhizae on competitive interactions among hosts (Crush 1974, Fitter 1977, Hall 1978, Janos 1981, Taylor 1982), however, and I know of no such studies for ectomycorrhizae. Whether or not mycorrhizal interactions can influence plant community composition when suitable symbionts are adequately provided is a question crucial to resolving the potential role of mycorrhizae and host/fungus interactions in higher-plant community dynamics.

I suggest that mycorrhizal interactions influence community composition by affecting competition among plants; mycorrhizal fungi are thereby more agents than symptoms of the occurrence of higher-plant species. I here present several testable hypotheses concerning the effects of mycorrhizae on competition in the lowland humid tropics. These hypotheses are broadly applicable if interpreted with respect to departure of conditions from those prevailing in the tropics that lead to pronounced effects of mycorrhizae on competitive interactions: i) the majority of tropical soils have little available phosphorus, hence many tropical plant species depend on mycorrhizae for survival and growth, ii) undisturbed lowland humid tropical soils are temporally constant (although not necessarily spatially uniform) because seasons are lacking and decomposition and leaching rates are generally high, hence host dependence on mycorrhizae likely does not vary, and iii) tropical plant communities are among the richest in species on earth with potentially intense competition among those species.

I propose that effects of mycorrhizal interactions on competition provide an explanation for the way in which many plant species coexist in tropical communities, a problem that has long perplexed biologists. Explanations of coexistence in multi-species communities are usually based upon either of two assumptions, but I will use both. Niche differentiation is most often assumed; it asserts that interspecific competition organizes communities by minimizing interactions among species through their evolution. This assumption requires a high degree of habitat heterogeneity to explain the coexistence of many plant species. An alternative assumption of synergism among species is frequently invoked by systems ecologists; it holds that emergent system properties resulting from complex interconnections or interactions among species convert potentially negative interactions into mutually beneficial ones at the community level. Among 72 published studies which searched for competition the reverse, synergism, occurred in 14 (Connell 1983), supporting this assumption. I argue that competition can be understood in the context of niche differentiation when i) between ectomycorrhizal and VA mycorrhizal species and among ectomycorrhizal species, ii) between non-mycorrhizal and mycotrophic species, and iii) among VA mycorrhizal species growing on fertile soil, but that synergism is likely to occur among VA mycorrhizal species on infertile soil.

Ectotrophs versus VA mycotrophs

Ectomycorrhizal hosts ("ectotrophs") are relatively rare in the tropics compared to their abundance in the north-temperate zone (Janos 1983). They occur i) at high elevations, ii) in "monsoon" forests with a distinct dry season, iii) on extremely poor soils (Spodosols), and iv) as seral species. Where they do occur they are often forest dominants (e.g. the dipterocarpaceae in Asia), or occur in very low diversity stands (e.g. some legumes in the Neotropics and Africa). I hypothesize that the distribution of ectotrophs in climax associations in the tropics results from a higher carbon cost of their fungus associates than that imposed by VA mycorrhizal fungi on their hosts; high mycobiont cost limits ectotroph occurrence to those habitats in which ectomycorrhizae are more beneficial than VA mycorrhizae.

Ectomycorrhizal fungi may require more photosynthate from their hosts than do VA mycorrhizal fungi from similarly dependent hosts for any of several reasons: i) there is a greater biomass of hyphae, mantle hyphae, mycelial strands and rhizomorphs, sclerotia, and sporocarps associated with ectomycorrhizae than of hyphae, spores and sporocarps with VA mycorrhizae, hence too a higher carbon cost if equal hyphal respiration rates of ectomycorrhizal and VA mycorrhizal fungi are assumed, ii) ectomycorrhizal fungi are a stronger sink for photosynthate than VA mycorrhizal fungi because

ectomycorrhizal fungi produce plant hormones that influence carbon-compound translocation, and they convert host sugars into storage sugars (in contrast to VA mycorrhizal fungi which probably use host photosynthate directly for respiration and growth), and iii) some ectomycorrhizal fungi may produce cellulase or polyphenol oxidase and in consequence may be expensive of energy for hosts to control (Janos 1983). Although ectomycorrhizae are usually considered to be an adaptation to infertile soils, the single fungus species associated with Neea laetevirens (Nyctaginaceae) in Costa Rica may be unable to extract sufficient mineral nutrients from a soil low in available phosphorus to amortize its cost to the host. N. laetevirens seedling growth was greater in the more fertile of two soils even though infection did not differ significantly between the two soils (Table 1).

Several morphological and physiological differences between ectomycorrhizae and VA mycorrhizae may impart advantages over VA mycotrophs to ectotrophs: i) storage of phosphorus in the mantle for subsequent release to the host buffers the host against fluctuations in phosphorus availability, ii) reduction of water loss, rapid rewetting, and protection from pathogens (the latter being especially important for an abundant host species) by the mantle lengthens root life, thereby increasing mineral uptake and retention, iii) transport of mineral nutrients over decimeters by mycelial strands and rhizomorphs allows nutrients to be extracted from a very large area, iv) utilization of ammonium and organic nitrogen compounds as nitrogen sources avoids dependence on nitrifying organisms (it is unclear whether or not VA mycorrhizal fungi can take up these compounds), v) litter decomposition by ectomycorrhizal fungi that produce cellulolytic or lignin-decomposing enzymes directly acquires mineral nutrients without loss to other decomposers, and vi) production of copious wind-dispersed spores confers rapid-colonization ability.

In consequence of their potential advantages over VA mycotrophs, I predict that ectotrophs are most likely to compete successfully with VA mycotrophs in those habitats in which: i) mineral nutrient and water availability is pulsed, ii) nitrification is inhibited, iii) the ability of the mineral soil to supply or retain mineral nutrients is very limited, iv) decomposition is slow (Gadgil & Gadgil (1971) suggested that the presence of ectomycorrhizae can slow decomposition; such an effect would favor only those ectomycorrhizal fungi which themselves are capable of mineralizing litter), and v) rapid colonizing ability of both symbionts favors establishment and persistence. These predictions are consistent with the presently known distributions of tropical ectotrophs.

Specificity of mycorrhizae and competition among ectotrophs

I hypothesize that ectomycorrhizal fungi compete far more strongly for root infection sites than do VA mycorrhizal fungi, and as a result, ectotrophs have greater opportunity to favor particular fungus associates than do VA

Table 1. Infection and growth of ectomycorrhizal seedlings* of Neea laetevirens in two soils.

Feature	Old Alluvium	Recent Alluvium
Available P (ppm)	6	12
No. of seedlings	12	22
Infection (%)	0.48	0.56
S.D.	0.116	0.134
Significance+	NS	
Height (cm)	8.17	14.35
S.D.	2.19	4.86
Significance++	$p<0.001$	

*Only seedlings with more than 30% of their ultimate roots infected are included.
+Difference assessed by t-test of untransformed percentages which fell between 30% and 81%.
NS = not significantly different at p=0.05.
++Difference assessed by approximate t-test assuming unequal variances.

mycotrophs. Such discriminatory ability may lead to specificity of association and might account for the occurrence of some tropical ectotrophs in monospecific stands. Exclusive specificity in which ectotroph species do not share mycobiont species could contribute to dominance by one or a few ectotroph species in two ways: i) temporal and spatial homogeneity of the soil would confer the greatest advantage in competition on the best adapted ectotroph, and ii) on extremely infertile soils, mineral limitation of mycobiont fruiting (which might occur because mycobionts that are unable to reproduce unless associated with a host must relinquish minerals needed for sporophore production to the host in order to insure host survival and a continued energy supply) could limit adequate mycorrhiza formation to only those host species that are sufficiently competitive to achieve a threshold abundance or close spacing needed for vegetative transmission of infection among individuals.

In order for specificity of mycorrhizal associations to evolve, hosts and/or mycobionts must be capable of discriminating and selecting among potential associates. VA mycotrophs are much less likely than ectotrophs to be able to favor a more effective over a less effective mutualistic fungus regardless of the order of infection because several VA mycorrhizal fungi can infect the same root tip. A single ectomycorrhizal fungus species usually infects an individual rootlet. Ectotrophs could favor a mycobiont by differential photosynthate translocation or by toxin production affecting single rootlets or portions of root systems. VA mycotrophs are required to discriminate among sets of cortical cells within single root tips. Discrimination at the cellular level by regulation of energy supply is not likely because root cells are not sources of energy compounds. Toxins could be produced within individual cells,

but this would require a mechanism for comparison of the relative effectiveness of mycobionts in different cells.

VA mycobionts are less likely than ectomycorrhizal fungi to specialize on single host species. Such specialization enhances the fitness of a mycobiont only if more resources are obtained through specialization; specialization must increase occupancy of the roots of the sole host sufficiently to compensate for decreased association with other plant species. Increased root occupancy probably requires exclusion of other mycobiont species. Vigorous ectomycorrhizal fungi can replace less vigorous fungus species, and after initial infection they may spread rapidly along roots; VA mycorrhizal fungi are much less able than ectomycorrhizal fungi to exclude one another, and their capacity for spread along root systems is limited.

In addition to the inabilities of hosts to discriminate among mycobionts and of mycobionts to competitively exclude one another, there may be other reasons why VA mycorrhizal associations lack specificity: i) environmental fluctuations might change the relative effectiveness of VA mycobionts such that no one is consistently superior (although I have suggested that the tropical soil environment fluctuates little), and ii) VA mycorrhizal infection might be highly unpredictable because of limited sporulation, spore dispersal, and vegetative spread such that neither hosts nor fungi can reject any mutualistic associate because encounter of an optimal partner cannot be anticipated. Although ectotrophs are capable of niche differentiation through specificity of association, VA mycotrophs do not appear to be similarly able. Lack of specificity among VA mycorrhizal associates may contribute to synergism in communities of VA mycotrophs.

Degrees of dependence on mycorrhizae

Competition between non-mycorrhizal and mycotrophic species and especially among VA mycotrophs is greatly influenced by the degrees of dependence on mycorrhizae of hosts. Both ectomycorrhizal and VA mycorrhizal host species can be ordered along a continuum of dependence on mycorrhizae for growth that ranges from facultative to obligate mycotrophy (Janos 1980). Although few species except perhaps some palms may be incapable of growth without mycorrhizae at all levels of soil fertility, many tree species may be ecologically obligately mycotrophic -- unable to grow without mycorrhizae in the most fertile soils naturally encountered. In contrast, infection does not improve the growth of facultative mycotrophs at high levels of fertility and is rejected. Independence of mycorrhizae at high fertility implies that the latter species possess adaptations for mineral nutrient uptake that are less expensive of energy than mycorrhizae. The most obvious of these is the presence of abundant root hairs, although presence of root hairs is not perfectly correlated with independence of mycorrhizae because other adaptations may substitute for both root hairs and mycorrhizae. Among non-mycorrhizal species, for example, Phytolacca

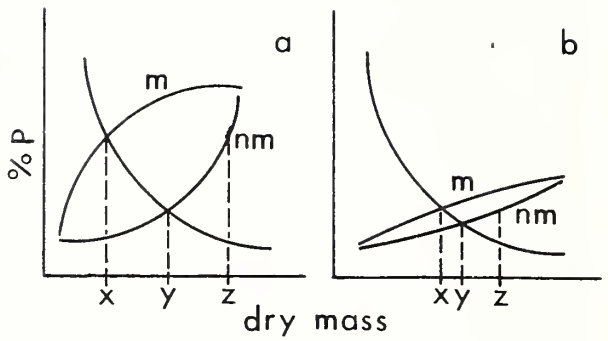

Figure 1. Hypothesized relationship between plant tissue phosphorus concentration (%P) and plant dry mass for facultative (a) and obligately mycotrophic species (b). Curves labeled "m" represent mycorrhizal plants, and "nm" curves represent uninfected plants; these are crossed by isopleths for P content. The "costs" of mycorrhizae assessed as reduced dry mass are indicated by distance along the horizontal axis from x to y for plants with the same total P, and from x to z for the same P concentration.

rivinoides lacks root hairs. Non-mycorrhizal species are completely independent of mycorrhizae (although infection may occur when they are senescent). Instead, they depend upon fine, highly-branched root systems, root hairs, or secretion of organic acids (which can solubilize phosphorus by lowering the pH of alkaline soils, and may release occluded phosphorus from clay aggregates in acid tropical soils (see Graustein, Cromack & Sollins 1977)), or they tolerate limited nutrient supplies by low tissue mineral requirements or by slow growth.

I suggest that among mycotrophic species obligate mycotrophs differ from facultative ones in a fundamental way which affects competition: the growth of obligate mycotrophs is mineral-limited, but that of facultative mycotrophs is most often energy-limited. Obligate mycotrophs cannot take up adequate mineral nutrients, especially phosphorus, for growth without mycorrhizae. Such species must sustain high levels of infection at all soil fertilities. If infection is regulated by root tissue phosphorus content through an influence on membrane permeability (see Graham, Leonard & Menge 1981), then immediate utilization for root and shoot growth of phosphorus supplied by mycorrhizae would favor continued infection. Stated another way, obligate mycotrophs should not accumulate "luxury" tissue phosphorus that might suppress infection. Facultative mycotrophs do accumulate luxury phosphorus; when uninfected by mycorrhizal fungi they can produce the same dry mass as is produced with a much higher tissue phosphorus content when infected (see Stribley, Tinker & Rayner 1980; Figure 1a). I predict that the tissue phosphorus content of obligate mycotrophs changes less with increasing dry mass than that of facultative mycotrophs, and that if uninfected individuals with the same dry masses as exhibited by infected individuals could be

produced (perhaps by injection), then infected and uninfected individuals of the same mass would differ very little in tissue phosphorus concentration (Figure 1b versus 1a). A consequence of these predictions is that the "cost" of mycorrhizae as assessed by Stribley, Tinker and Rayner (1980) is lower for obligate than for facultative mycotrophs, i.e., the utilization of photosynthate to sustain mycorrhizae reduces the growth of obligate mycotrophs less than that of facultative mycotrophs.

Non-mycorrhizal versus mycotrophic species

Janos (1980) predicted that greatest competitive success of a majority of non-mycorrhizal species can be expected under infertile conditions when the mycorrhizal fungi required by mycotrophic species are lacking. Here I consider the competitive success of non-mycorrhizal species when suitable symbionts are adequately available to mycotrophic species. I hypothesize that non-mycorrhizal species can outcompete mycotrophic species in the presence of mycorrhizal fungi under any of three conditions: i) if there is a lag in the onset of rapid growth of mycotrophic species because of slow infection, ii) if the non-mycorrhizal species secrete allelopathic chemicals effective against mycorrhizal fungi thereby reducing the probability of infection of mycotrophs (a hint of this possibility comes from Chinese herbal medicine which uses a root decoction of a Phytolacca species against dermatophytic fungi), or iii) if the habitat-influenced benefit/cost ratio of the mineral uptake adaptations of non-mycorrhizal species exceeds the benefit/cost ratio of mycorrhizae to mycotrophic species.

A corollary of the third condition listed above is that non-mycorrhizal species may better compete with facultative mycotrophs than with obligate mycotrophs because of the sensitivity of facultative mycotrophs to energy limitation (i.e. at any level of fertility at which infection is sustained the benefit/cost ratio of mycorrhizae for a facultative mycotroph is likely to be exceeded by those of obligate mycotrophs). In the presence of mycorrhizal fungi, non-mycorrhizal species are more likely to occur at intermediate levels of fertility than to compete effectively with obligate mycotrophs at low fertility. The tropical forest studied by St. John near Manaus, Brazil in which adult individuals of several abundant species of Lecythidaceae are non-mycorrhizal on an infertile soil (St. John 1980), however, may contradict this prediction. It would be interesting to know if those species are abundant because their seedlings are obligately mycotrophic even though adults are non-mycorrhizal, or alternatively, if they are abundant because the prevalence of non-mycorrhizal adults is self-reinforcing through lowered probability of infection of mycotrophic seedlings as suggested by Janos (1980, 1983).

Competition among VA mycotrophs

The outcomes of competition among VA mycotrophs depend upon soil fertility and upon the degrees of dependence on mycorrhizae of competing host species. In the absence of mycorrhizal fungi, the more facultatively mycotrophic of two competing species is likely to be the best competitor provided that there are adequate mineral nutrients available to permit its growth. In the presence of abundant VA mycorrhizal inoculum, however, the possible outcomes of competition are more complicated. Three combinations of species that differ in degree of dependence must be considered for both fertile and infertile soils. These combinations are facultatively mycotrophic species, facultative and obligate mycotrophs, and obligate mycotrophs. Although degrees of dependence on mycorrhizae form a continuum, these three combinations are discrete because ecologically obligate mycotrophs are distinguished from facultative mycotrophs by failure to grow without mycorrhizae at any level of soil fertility naturally encountered. Over an ecologically relevant range of soil fertilities, ecologically obligately mycotrophic species cannot be distinguished from one another as differing in dependence on mycorrhizae.

I base hypotheses concerning the outcomes of competition among VA mycotrophs on two assumptions: i) the energy cost of mycorrhizae most retards the growth of the least mycorrhiza-dependent of two competing species, and ii) if neither of two competing species rejects mycorrhizal infection, then one or both may experience greater infection than when growing alone. The first of these assumptions is a consequence of my argument that the relative cost of VA mycorrhizae decreases with increasing dependence on mycorrhizae; the second is supported by the results of Fitter (1977) who observed that the poorer competitor of two competing grass species sustained greater infection than when grown alone. Supra-optimal infection of a less-dependent competitor might be attributable to a more-dependent "donor" increasing the supply of energy to its mycorrhizal fungi which then become more competent to infect the competitor (see Francis & Read 1984).

When two facultative mycotrophs or a facultative and an obligate mycotroph are competing, I predict that if soil fertility is not sufficiently high for the least mycorrhiza-dependent competitor to reject infection, then the most mycorrhiza-dependent species will be the best competitor. The strongly dependent species will foster supra-optimal infection of its competitor, increasing the energy cost of mycorrhizae to the competitor and thereby reducing its growth. This assumes that the competitor cannot "parasitize" the donor by living in partial heterotrophic dependence on it (but see Francis & Read 1984). If soil fertility is sufficiently high for the least mycorrhiza-dependent species to reject infection, then that species will be the best competitor because it does not sustain the energy cost of mycorrhizae. Notwithstanding these hypothesized competitive superiorities, both species may coexist to the extent that niche overlap and complete competitive exclusion are avoided because of differing ecologies

(especially different mineral nutrient requirements, different rooting zones, and greatly different dependence on mycorrhizae).

When competing species are very similar in dependence on mycorrhizae and that dependence is great, i.e. when both species are obligately mycotrophic or soil fertility is very low (but not so low as to preclude a mycotrophic response), abundant infection of competitors by the same mycorrhizal fungi may eliminate differences among hosts in ability to compete for mineral nutrients. The probability of their coexistence thereby may be greatly increased. I suggest that synergism is a consequence of niche convergence on mineral nutrition niche axes which is caused by extreme dependence of host species on mycorrhizae and by the lack of specificity of VA mycorrhizal fungi. If the ability of host species to take up minerals from the soil is entirely that of their associated fungi, and if they are associated with the same fungus species, then mycorrhizal interactions tend to reduce differences among host species in ability to compete for mineral nutrients. If mineral nutrient supply is the major constraint on species occurrence, as it may be in tropical forests, then mycorrhizal interactions increase the probability of coexistence of many species.

The results of an experiment that I conducted in Panama (Janos 1981; Table 2) demonstrate that VA mycorrhizae can favor species coexistence. Although mycorrhizal inoculation of nine species competing in microplots more than tripled above-ground biomass production which contributed to increased competition, inoculation more than doubled the number of surviving individuals of four of those species. Fertilization in the absence of mycorrhizal fungi did not have a similar effect, suggesting that mycorrhizal interactions of the sort that I have postulated and not simply augmented mineral availability contributed to species coexistence.

Conclusions

I have argued that mycorrhizal interactions influence community composition by affecting the competitive abilities of plants, not merely by lack of suitable mycobionts limiting the occurrence of dependent species. Niche differentiation with respect to mineral nutrition can be effected by i) dependence on different types of mycorrhizae, ii) exclusive dependence on specific mycobionts, or iii) independence of mycorrhizae arising from other adaptations for mineral uptake or high soil fertility. Outcomes of competition among species that have thus differentiated (but for which niches still overlap somewhat) may depend in ways which I have suggested upon the availability of suitable mycobionts, water, and different forms of mineral nutrients. Synergism -- facilitative interactions among species -- may result from mycorrhizal interactions among hosts that are highly dependent on non-specific, shared mycobionts in infertile soils. Synergism favors the coexistence of species, and may partially account for overyielding of polycultures in tropical agriculture and for high within-habitat species diversity in tropical forests.

Table 2. Performance of nine species of tropical trees competing in microplots. -MYC denotes uninoculated plots, +MYC denotes plots inoculated with VA mycorrhizal fungi, and +NPK denotes plots receiving 1 kg of 12-24-12 fertilizer.

Treatment	Mean # ind./ plot	Mean tot. above gnd. dry matter (g)	Total individuals of 4 spp. *
-MYC	35	443	29
+MYC	39	1441	72
-MYC+NPK	31	8968	30

* Mycorrhizal inoculation at ambient fertility significantly improved the survival of Bixa orellana, Cordia alliodora, Ficus obtusifolia, and Luehea semanii; survival of other species was unaffected.

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MYCORRHIZAE IN TEMPERATE COMMUNITIES: MAXWELL'S ECOLOGICAL DEMON

By

David A. Perry

"They tell me there are leucocytes in my blood and sodium and carbon in my flesh. I thank them for the information and tell them there are black beetles in my kitchen, washing soda in my laundry, and coal in my cellar. I do not deny this existence, but I keep them in their proper place."

G.B. Shaw, Back to Methuselah

Abstract

Ectomycorrhizal fungi (EMF) occupy different niches within the ecosystem and have differential adaptations to disturbance and varying sensitivity to soil and litter chemistry. This differentiation may increase the environmental responses available to the higher plant community, in particular increasing ecosystem resilience after major disturbance and decreasing rooting zone competition. If true, EMF could be thought of as ecological analogues to James Clerk Maxwell's imaginary "Demon" that imposes a high degree of order on a system with relatively little energy cost.

Keywords--Ectomycorrhizae, competition, stability, resilience.

Introduction

Mycorrhizal symbiosis is by definition an ecological phenomenon, and much research has been devoted to mycorrhizae as a critical link in the nutrient cycle. We now realize that mycorrhizal fungi are likely to be much more than semipermeable membranes for individual plants, that they may play key roles in the structure and function of ecosystems (see Harley and Smith 1983, Chapt. 19). Their role in carbon cycling has been the subject of much recent research (e.g., Gadgil and Gadgil 1975; Fogel and Trappe 1978; Fogel and Hunt 1979; Vogt et al. 1981, 1982), and, while evidence is for the most part circumstantial, there is growing conviction that mycorrhizal fungi mediate competitive interactions between plants and thereby influence the temporal and spatial structure of ecosystems (Bowen 1980; Perry, in press; Janos, this volume; Reeves, this volume).

Herein I discuss the possible roles of ectomycorrhizal fungi (EMF) in ecosystem recovery after disturbance, and in resource allocation between individual plants and therefore in primary productivity of ecosystems. The underlying theme is that EMF species occurring on a given site are adapted to different niches and that this differentiation multiplies the environmental responses available to the higher plant community. Thus, EMF perform a role somewhat analogous to that imaginary "Demon" which the 19th century physicist James Clerk Maxwell suggested could violate the second law of thermodynamics by imposing order on a system with very little energy cost. I deal exclusively with EMF in temperate systems; tropical systems and endomycorrhizae in temperate systems are discussed respectively by Janos and Reeves in this volume. In keeping with the intent of the conference, I largely speculate about what might be, rather than review what is.

EMF in System Recovery After Disturbance

In a recent theoretical paper, Roberts and Tregonning (1980) show that stable systems are characterized by a large number of overlapping subsystems which are themselves stable, and they speculate that gross perturbation of natural systems reduces the parent system to one or more of the stable subsystems. It follows that the surviving subsystems may serve as a base for the parent system to reconstruct itself. In temperate forests, many of which are characterized by periodic catastrophic disturbance, soil subsystems are often relatively stable and serve as focal points for reorganization of the parent system. Although many pioneer plants are non- or facultatively mycorrhizal (Reeves, this volume), successful recolonization of severely disturbed areas (e.g., mine spoils) may require mycorrhizal fungi, and in most, if not all, cases, the process of recovery includes replacement of plants that do not need mycorrhizae by those that do. It is probably safe to assume, then, that mycorrhizal fungi are essential at one stage or another of system reorganization after disturbance, and that they are therefore either relatively stable after major perturbation or efficient recolonizers.

I hypothesize that stability of the EMF community is related to its species diversity. Evidence for this view comes from a series of studies comparing ectomycorrhizal (EM) formation on tree seedlings grown in soil from disturbed (clearcut) and adjacent undisturbed sites (Perry et al. 1982; Schoenberger and Perry 1982; Pilz and Perry 1984; Perry and Rose, in press). Figure 1 shows the relationship between EM diversity in undisturbed soils and the change in EM formation with disturbance. Diversity is indexed according to the Shannon formula (Pielou 1969), which takes into account both richness and evenness (higher values indicate greater diversity). EM types are

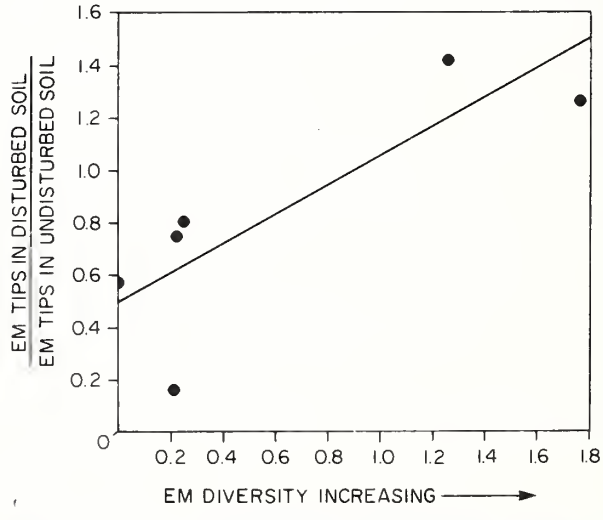

Figure 1. Relation between the effect of disturbance on EM formation and EM diversity ($-\Sigma\ p_i\ \ln\ p_i$) in undisturbed soils.

distinguished by morphology and may or may not correspond to different species. Change in EM formation is expressed as the proportion of EM tips formed in disturbed relative to undisturbed soils. Low EM diversity, and reduction in total EM after disturbance, occurs in high-elevation lodgepole pine types in southwest Montana and relatively droughty habitats in the Siskyou Mountains of southwest Oregon, while sites with high EM diversity and increased EM formation after disturbance are from mesic, productive habitats in the Oregon Cascades. Note that, on these sites, EM diversity is clearly correlated with (a) site productivity, and (b) efficient EM formation after disturbance.

The mechanism behind the buffering action of EM diversity seems to be very simple--different EMF are adapted to different conditions. In the Oregon Cascades there were highly significant interactions between EM type and disturbance class--the change in proportion of various types depending on whether seedlings were grown in undisturbed, logged and burned, or logged and unburned soils (Schoenberger and Perry 1982; Pilz and Perry 1984). As with higher plants, there appear to be early and late successional EMF (cf. Mason et al. 1983).

Numerous factors may determine success of a given EMF species in a particular environment, including competition with other EMF species, light levels (Pilz and Perry 1984), and soil-litter chemistry (Schoenberger and Perry 1982; Rose et al. 1983; Gardner and Malajczuk, this volume). I shall discuss the latter factor in more detail in the following section.

EMF in Resource Allocation Among Higher Plants

Inhibition of EM formation by water-soluble organic compounds has been demonstrated by Handley (1963) and Schoenberger and Perry (1982). Rose et al. (1983) showed that the effect of litter leachates on EMF growth in pure culture was highly dependent on species, litter type, and leachate concentration. In temperate forests, which accumulate more litter and stable soil organic matter than tropical forests, background biochemical levels should be generally high near the soil surface and decrease with depth. Because different EMF species respond differentially to type and concentration of litter leachates, a vertical biochemical gradient is likely to impose a vertical gradient in EM types. When EMF are not randomly distributed among plant species, this gradient would result in a vertical stratification of rooting zones among higher plant species. For example, when Douglas-fir and western hemlock seedlings are grown in the same soils, a relatively high proportion of Douglas-fir EM are Rhizopogon vinicolor, while a high proportion of western hemlock EM are Cenococcum geophilum. Growth of R. vinicolor is reduced by high concentrations of litter leachates of certain types, while that of C. geophilum is stimulated. It follows that when these tree species are grown together in the presence of litter or organic layers, western hemlock may root primarily in the organic layers, while Douglas-fir roots lower. Note that this differentiation is a function of the EMF and the litter type, not tree species. A tree species that forms EM with several fungal species will have numerous potential rooting behaviors. Thus, this mechanism could also decrease rooting competition among trees of the same species (if, for example, individual trees by chance formed different proportions of EM types).

During the recovery phase after disturbance, buildup of litter and the consequent intensification of the soil biochemical environment produce changes in the EMF types of individual trees (Gardner and Malajczuk, this volume). In temperate forests, rooting zones often shift from mineral soil to litter and humus layers during stand development (Cole 1981). This change may correspond to, and perhaps even result from, a shift from litter-sensitive to litter-stimulated EMF (e.g., from Pisolithus tinctorius to Cenococcum geophilum). It is reasonable to hypothesize that EMF species that are insensitive to, or stimulated by, litter leachates are those that produce enzymes, such as amidases, phenolases, or mixed-function oxidases, which break down or otherwise detoxify inhibitory organic molecules.

This scenario can be carried one step further. Suppose that EMF directly alter foliage chemistry. A closed loop would then exist in which EMF, rather than being passive prisoners of soil biochemistry, would be major actors in creating particular soil environments. Most of this is pure speculation; however, EM do alter foliage constituents other than simple nutrients. Figure 2 shows

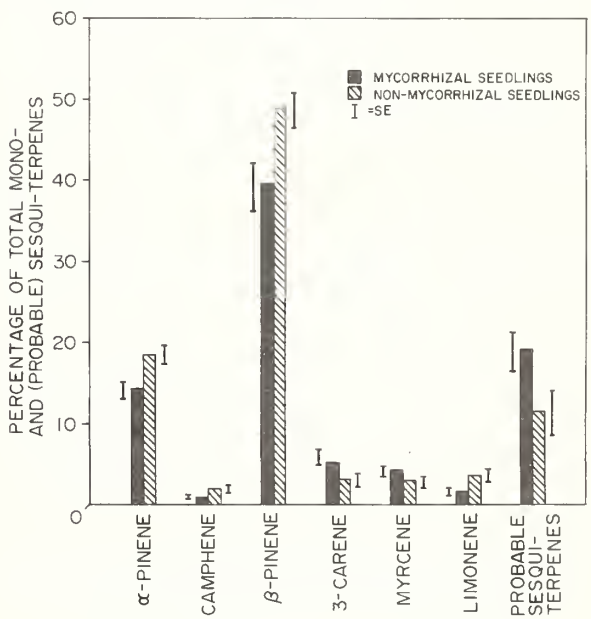

Figure 2. Influence of EM on Douglas-fir terpenes. Percentages are averages for seedlings infected with Hebeloma crustiforme and Cenococcum geophilum. Terpenes are arrayed (left to right) in the order in which they came off the gas chromatograph column. Monoterpenes were positively identified. The last group (furthest right) are probably various sesquiterpenes (G. B. Pitman, pers. commun.) but were not positively identified.

the influence of EM on the proportion of various terpenes in foliage of Douglas-fir seedlings (Perry, unpublished). The two EMF, Hebeloma crustiforme and Cenococcum geophilum, did not differ in their effect, and are averaged together. All mycorrhizal-nonmycorrhizal differences shown are significant at the 10 percent level or better (terpenes not significantly influenced by EM are not shown). EM clearly result in increased proportions of some terpenes, particularly the group which, although not positively identified, is composed of what are probably sesquiterpenes (G.B. Pitman, personal communication).

Sharon Rose and I (unpublished) have also found high-affinity ferric iron chelators, called hydroxymate siderophores (HS), in Douglas-fir foliage. These chelators are produced by microbes, including EMF, and it is possible that their presence in foliage is due to EMF, though we do not yet know that for sure.

The implications of these biochemical changes on litter decomposition, soil biochemistry, and EMF are unknown. Both sesquiterpenes and HS, however, may be important as herbivore and pathogen defenses. Because herbivores transform recalcitrant organics in foliage to compounds that are more readily decomposable, alterations of herbivory as a result of EMF could indirectly influence chemical transformations in litter and soil. Further, it seems reasonable that foliage constituents that affect herbivores and pathogens would also have a direct influence on decomposers (as we know is true for polyphenolics). Therefore, the elements seem to be in place for the hypothetical loop: EMF → foliage chemistry → litter chemistry → EMF; whether it actually exists, and has ecological significance, remains to be seen.

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EFFECTS OF ENVIRONMENT ON MYCORRHIZAE: LEAVING THE DARK AGES

By

Jennifer L. Parke

Keywords--soil factors, techniques, water stress, presence vs. activity, physiological indicators, native fungi

Fundamental to our use of mycorrhizae to improve plant growth is an understanding of how mycorrhizae are affected by environmental factors such as climate, soil chemical and physical factors, and biotic effects. Mycorrhizal fungi occur in a large diversity of plant communities and their adaptation to extremes of environment is widely acknowledged; at the same time, we are still in the "Dark Ages" with regard to applying these features of ecological specialization to agriculture, forestry, or in restoring native vegetation. In spite of enormous interest in "tailoring" host-fungus combinations for specific planting sites, we are limited by our understanding of the environmental characteristics of the site, the range of tolerance of the mycorrhizal symbiont, and the ability of the fungus to benefit the host under natural conditions. What are some of the experimental approaches and pitfalls in conducting research on environmental factors affecting mycorrhizae, and what are some of the recent advances in this field?

Traditional approaches in testing environmental effects on mycorrhizae include 1) a natural history phase of observation and correlation 2) field studies in which a response may be correlated with one or more measured variables and, 3) controlled environment experiments. Ectomycorrhizal fungi can also be tested in vitro, but results are generally found to have little relevance to the symbiotic association where environmental effects on the fungus are mediated by the host. Often, however, the limits of environmental tolerance are discovered accidentally when mycorrhizae fail to become established; unfortunately this trial and error method contributes little to our knowledge of why the failure has occurred.

Environmental factors can be tested individually or in combination using host-fungus bioassays in natural or artificially inoculated soil. We know little about the "epidemiology" of mycorrhizal infections, that is, the relationship between propagule numbers, rate of infection, spread, propagule formation and the relative importance of various propagules. This information is especially lacking for ectomycorrhizal fungi. Nevertheless, it is important to recognize that individual phases of the fungus life cycle respond differently to environmental effects; for instance, high temperatures which prevent colonization may not affect survival of dormant propagules (Parke, Linderman, and Trappe, 1983). Relatively few studies have dealt with mixed populations of mycorrhizal fungi to determine relative competitive ability under specific environmental conditions, but recent developments in identification of mycorrhizal species based on infection morphology and immunoassay techniques may allow more studies of this nature. Additional problems in mycorrhiza research arise in attempting to differentiate between effects on the plant and on the fungus; for example, lack of mycorrhizae at low temperature can be a result of reduced fungal growth or limited root growth (thus fewer potential colonization sites). It is often difficult to vary individual environmental factors; for example, a reduction in soil water will affect nutrient availability and care must be taken in interpretation of results.

There are technical problems associated with choosing what to measure and when to measure it. Measurement of a single parameter such as percent root length (VA) or percent root tips colonized (ectomycorrhizae) at a single point in time is generally inadequate for assessing response to an environmental variable. Other parameters, such as the amount and distribution of external hyphae, may be more relevant measures of mycorrhiza response and effectiveness. When comparing mycorrhizal and nonmycorrhizal treatments, it is especially important to have multiple harvest data. Mycorrhizal plant development may be accelerated as a result of an increased rate of nutrient uptake and more rapid depletion of resources in a small container when compared to nonmycorrhizal treatments (Fig. 1).

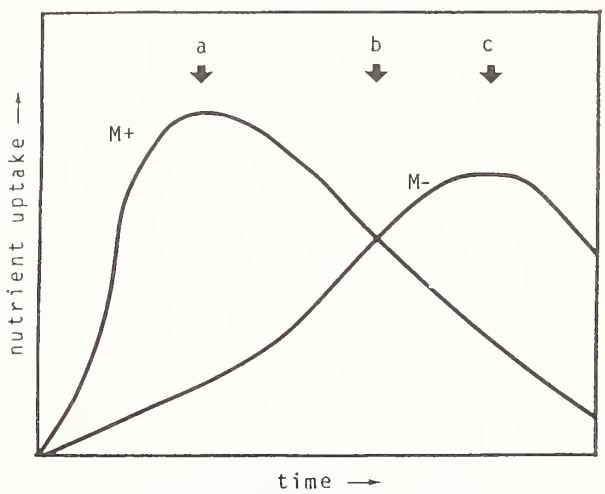

Figure 1. A single harvest of mycorrhizal (M+) and nonmycorrhizal (M-) treatments at arbitrary time a, b, or c can give misleading results. Multiple harvests are one way of overcoming differences in plant development as a result of the mycorrhizal condition.

Another sampling problem is illustrated in Fig. 2, in comparing the relative abundance of mycorrhiza propagules occurring in two sites (disturbed and undisturbed). Infectivity bioassays conducted using soil samples collected once at time a or b can yield disparate results. Seasonal fluctuation of propagule numbers should be determined before selecting a

sampling date.

We are aware of some of the environmental factors which influence mycorrhizae, but we are still in the Dark Ages regarding the specifics. For instance, in southwest Oregon, soils from old clearcuts contain fewer propagules of ectomycorrhizal fungi than adjacent undisturbed forest sites. There is a further reduction in propagule numbers in soils from clearcuts which were also burned to remove slash debris (Parke, Linderman, and Trappe, 1984). However, we do not know why. Many changes are associated with logging disturbance, including: physical disruption of fungal mycelium and mycorrhizal roots, removal of host plants and ingress of nonhost plant species, changes in light, soil temperature, soil physical and chemical properties, organic matter and associated microbial populations. So, differences in mycorrhizal populations observed between the two sites are the net effects of multiple components operating together over time.

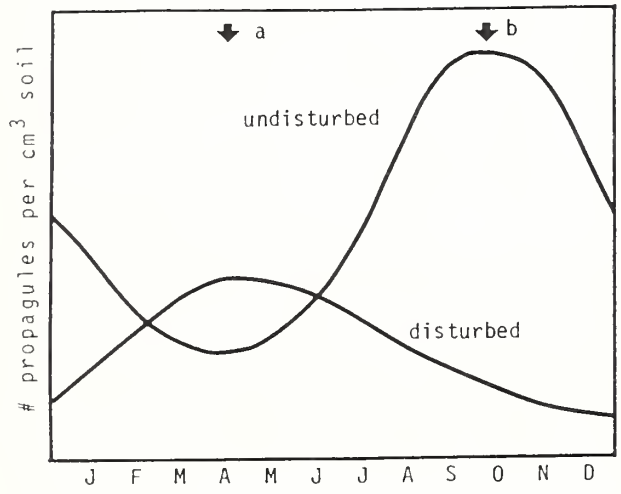

Figure 2. Soil collected once at time a or b could result in misleading infectivity bioassays as a result of differences in plant cover and patterns of resource depletion between disturbed and undisturbed communities.

There has been a surge of interest in native mycorrhizal populations as affected by disturbance associated with mining, cropping, and deforestation. Indigenous fungi might be expected to have a broader environmental tolerance range than certain introduced mycorrhizal fungi, and should be tested for their potential to improve plant growth. In comparing the merits of native and introduced strains of mycorrhizal fungi, it is important to perform the experiments under conditions which best simulate the field situation; native fungi may be less effective than other strains under greenhouse conditions, however, native species may outperform introduced species under conditions of environmental stress. A native ectomycorrhizal isolate from Oregon did not significantly increase growth of Douglas-fir when compared to nonmycorrhizal seedlings when both treatments were watered daily. However, when seedlings were watered only every fifth day, the native isolate significantly increased needle area, root and shoot dry weight compared to the nonmycorrhizal controls (Parke, Linderman, and Black, 1983). The criteria for selecting the appropriate fungal symbiont should be carefully considered; although increased P uptake is an important attribute, this may not be the factor most limiting to plant growth on many sites. For example, the leading cause of first-year mortality of conifer seedlings planted in southwest Oregon is plant moisture stress. Here, the ability to increase host tolerance to drought should be the primary criterion for symbiont selection.

In conducting research on mycorrhizae, it is important to distinguish between presence or abundance of the fungus vs. activity, or actual benefit to the host. Plant physiological indicators are useful in determining if mycorrhizae change host response to environmental stress. For example, the effect of water deficits on plant photosynthetic rate is easily demonstrated. Photosynthetic rate can thus be used to compare the response of drought-stressed mycorrhizal and nonmycorrhizal plants. Transpiration rate, dark respiration, and stomatal conductivity can be similarly measured and compared. These methods have the added advantages that they are 1) non-destructive, enabling sequential measurements on an individual plant throughout the duration of an experiment 2) measurements can be made in situ, in the growth chamber or in the field (Fig. 3), and 3) in combination with other measurements can often indicate the possible physiological mechanism(s) involved.

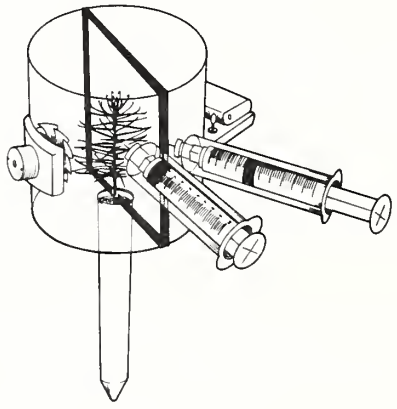

Figure 3. Example of a simple device for measuring CO_2 fixation in situ (Parke, Linderman, and Black, 1983).

Mycorrhizal fungi are just one component of a very complex rhizosphere community. They must compete with other soil fungi, actinomycetes, nematodes, and ameobae, and this ability to compete is modified greatly by environment. Their tolerance to environmental factors in pure culture is very different from their response in natural soil, thus it is important to include a suitable "background" microflora in predictive

assays for mycorrhizal effectiveness under a particular set of environmental conditions.

There are numerous technical difficulties associated with simulating environmental conditions or artificially imposing environmental stress on mycorrhizal and nonmycorrhizal plants. To solve these problems, mycorrhizae researchers are well-advised to collaborate with plant physiologists, soil chemists and physicists in designing, implementing, and interpreting their research. Only in this way will significant progress be made towards understanding how environmental variables affect mycorrhizal fungi and allow for more successful implementation of mycorrhiza technology.

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SURVIVAL OF VA MYCORRHIZAL FUNGI—INTERACTIONS OF SECONDARY SUCCESSION, MYCORRHIZAL DEPENDENCY IN PLANTS, AND RESOURCE COMPETITION

By

F. Brent Reeves

Keywords—ruderals, competitors, stress-tolerants, growth isoclines, nutrient levels

Mutualism, involving VAM fungi, is the most common state of affairs in higher plants; however, there has been a conspicuous lack of attention to mutualism in ecological theory and practice (May, 1984). Williams (1966) suggested that mutualism can evolve only if conditions are such that it is advantageous for the individual involved. If a species contains both mutualists and nonmutualists then the mutualists potentially benefit another species in its vacinity, but at a cost to itself. It has been argued that the proportion of mutualists must decrease in any group containing both mutualists and nonmutualists, because the mutualists bear a cost while the entire group benefits. However, groups with many mutualists will, because of the benefits derived, produce more offspring than those with few mutualists. This condition creates a force favoring mutualism. In general, mutualism will be favored by a small cost, a large benefit, and a small number of individuals. These ideas have been partially quantified using a mite-beetle association (Wilson, 1983).

Can such concepts be applied to VAM associations? Basic to an understanding of the nature of this mutualism are such questions as the cost, C, the benefit, B, and the population size, N. Survival of VAM fungi and the ubiquity of the association is predicated on low relative cost to the host and a positive net benefit to the mutualists.

Abundant research has shown that VA mycorrhizal fungi increase nutrient, including phosphorus and nitrogen, and water uptake in certain environments where essential nutrients are limiting. The magnitude of these "benefits" frequently are not measured. But what is sometimes called "mycorrhizal dependency" gives clues as to the magnitude of the benefit for a certain soil nutrient level. In essence, mycorrhizal dependency is expressed as the difference in growth, measured in several different ways, between a non-mycorrhizal and a mycorrhizal example of the same species grown at a particular available nutrient level. Very often the nutrient that is examined is phosphorus.

Typically plants fall into three general categories with regard to their VAM relationships. There is a continuum of plant species that range from those that are never mycorrhizal (M-), to those that are facultatively mycorrhizal (M±), to those that are obligately mycorrhizal (M+) under natural conditions. Those plants that are, for all intents, never mycorrhizal are annuals and weedy species. The facultatively mycorrhizal species may include those plants that are heavily colonized by VAM fungi when soil available phosphorus is low but not heavily colonized when soil phosphorus is high. And the obligately mycorhizal species are those plants that must have mycorrhizae regardless of the phosphorus level. This continuum of increasing mycorrhizal dependency may be represented in the field in certain situations where secondary succession occurs. Using the plant life strategy concepts of Grime (1977), secondary succession on heavily disturbed, semiarid soils begins with ruderals (M- species), proceeds to competitors (M± species), and the "climax" community is characterized by stress-tolerants (M+ species). These relationships are given in Fig. 1 and are supported by similar successional sequences in the tropics (Janos, 1980).

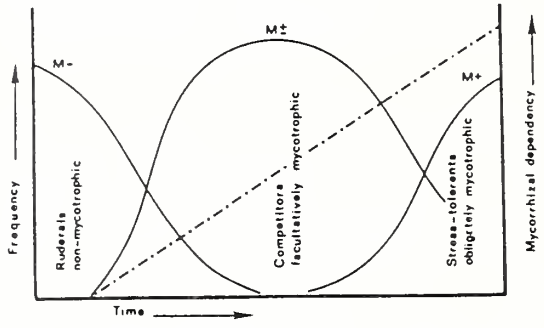

Fig. 1. A hypothetical sequence for succession based on mycorrhizal relationships of plants. Early successional stages are non-mycorrhizal (M-), intermediate stages are facultatively mycorrhizal (M±), and late stages are obligately mycorrhizal (M+). As succession proceeds, mycorrhizal dependency increases.

The interrelationship of Stress and Disturbance (Grime, 1977) determines the types of plants found in an ecosystem, Fig. 2.

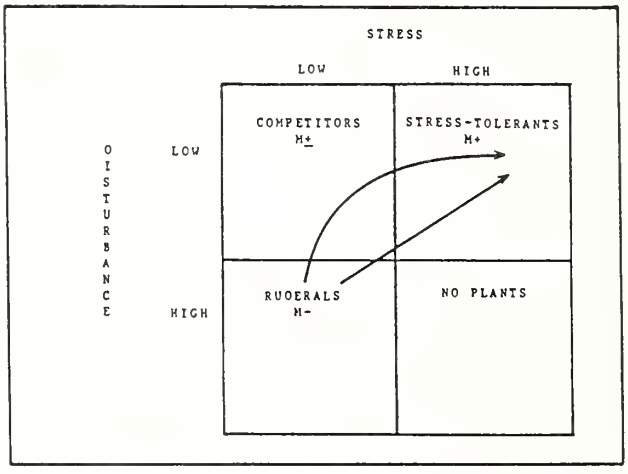

Fig. 2. The relationship of primary plant strategies to Stress and Disturbance. Ruderals are non-mycorrhizal (M-), Competitors are faculatatively mycorrhizal (M±), and Stress-tolerants are obligately mycorrhizal (M+). Succession is indicated by the heavy arrows.

There appears to be a potential correlation between the survival of mycorrhizal fungi and the hosts present on the Stress/Disturbance matrix. If Disturbance remains great, weedy species persist. As Disturbance is reduced, succession can proceed either directly to stress-tolerant species or through

competitor species to stress-tolerant species. In either case, there is the development of plants that are able to host mycorrhizal fungi.

For the facultatively mycorrhizal species (M±), mycorrhizal dependency is a relative term. A factor that must be considered when assaying the degree of colonization of the host plant is the relative efficiency of the fungus in acquiring soil nutrients. Efficiency is a function of ramification of the fungus in the soil, efficiency of nutrient exchange, and efficiency of nutrient uptake by the fungus. Thus experimental methods designed to acquire information on the "benefit" of the fungus should initially involve a single strain of the fungus and host.

Mycorrhizal dependency has a profound theoretical potential for explaining competitive exclusion of selected plant species growing in soils with low available phosphorus levels.

In certain ecosystems, the level of available phosphate decreases during secondary succession (Odum, 1969). Under such conditions, the plants that will survive will be those better able to compete for available phosphate. Thus there is a selection pressure for the presence of mycorrhizal symbionts. The non-mycorrhizal ruderals first will be replaced with facultative mycotrophs, and the facultative mycotrophs will be replaced with obligate mycotrophs as the level of phosphorus decreases. The "climax" vegetation will be stress-tolerants and obligately mycorrhizal.

One of the major "climax" communities of western Colorado is the pinyon-juniper formation. Pines must be mycorrhizal to survive. Our research has shown that one of the native junipers, Juniperus osteosperma, exhibits significant growth enhancement when mycorrhizal and is probably obligately mycorrhizal. Thus the pinyon-juniper formation can be characterized as a "stress-tolerant", obligately mycorrhizal "climax" community. This "climax" community satisfies Grime's (1977) concepts for plant stratigies and Janos' (1980) concepts for mycorrhizal dependency.

It appears that the survival of VAM fungi is closely correlated with the survival "benefits" conferred on the higher plants. As succession proceeds, the obligatory, symbiotic relationship of the higher plants with the VAM fungi increases. By using Tilman's (1982) ideas on resource competition in homogeneous environments, a hypothetical, graphic representation of the potential growth of selected species in a plant community can be formulated, Fig. 3. When two resources are essential (neither substitutable nor switching) and a species' growth equals its mortality rate, a Zero Net Growth Isocline (ZNGI) is established. The position of the ZNGI for a particular species is dependent on the resource requirements for that species. In general, the population density of a particular species will increase if the resource levels exceed the minimum level needed for growth and reproduction. Since most plants grow in communities that are heterogeneous (rather than homogeneous) in terms of nutrient availability, this representation is simplistic. However, Tilman's basic concepts can be employed to include nutrient competition among plants as modified by mycorrhizal fungi.

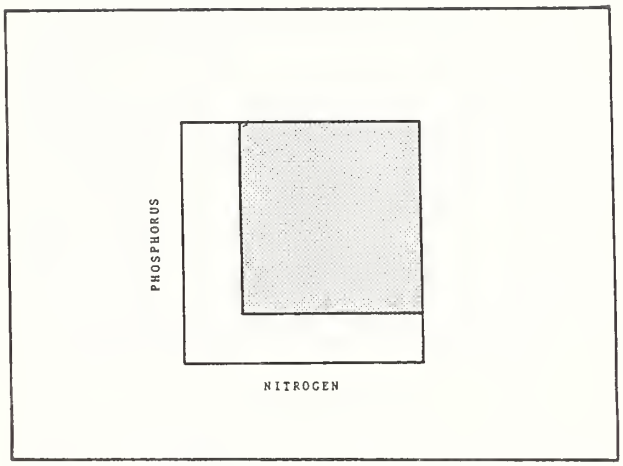

Fig. 3. The solid lines are the Zero Net Growth Isocline (ZNGI) for a particular species. In this example, the ZNGI represents the minimum level of available phosphorus and nitrogen at which net growth will occur. If greater amounts of resources, the shaded region of this graph, are available population size will increase.

When a species becomes mycorrhizal the available phosphorus level at which it can survive is effectively reduced. The ZNGI pattern is changed, i.e. there is a greater range of phosphate levels at which net growth will occur, Fig. 4.

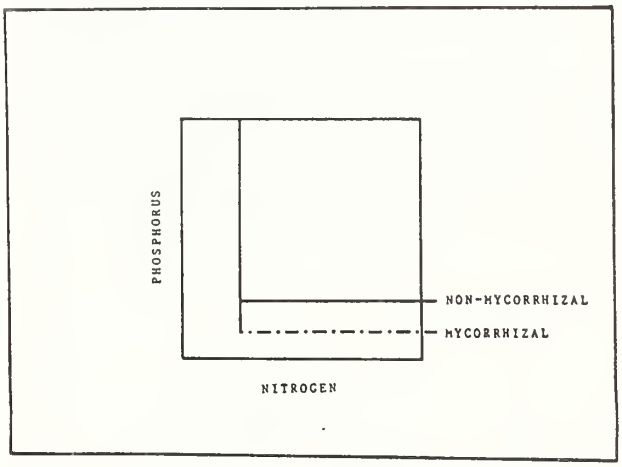

Fig. 4. Zero Net Growth Isocline for a hypothetical plant species. When non-mycorrhizal the species requires a higher available phosphate level than when colonized by mycorrhizal fungi.

When a single resource, say phosphorus, is considered, a graphic representation of the potential growth of the same species can be established, Fig. 5. At a low phosphate level, R_{M+}, the non-mycorrhizal plant will not grow as rapidly as the mycorrhizal plant. Mycorrhizal dependency, "the degree to which a plant is dependent on the mycorrhizal condition to produce its maximum growth or yield at a given level of soil fertility" (Gerdemann, 1975), can be estimated by the difference in phosphate levels at which one-half (r/2) maximum growth rate is achieved for both plants (R_{M-}-R_{M+}). Or mycorrhizal dependency can be defined as the difference in growth rates

between a non-mycorrhizal and a mycorrhizal plant growing at the same phosphate level (R_{M+} or R_{M-}).

The potential growth rate for a species can change with mycorrhizal colonization (Fig. 5); competitive exclusion of a non-mycorrhizal species may occur if one of several individual plants is mycorrhizal or becomes colonized by VAM fungi during its development.

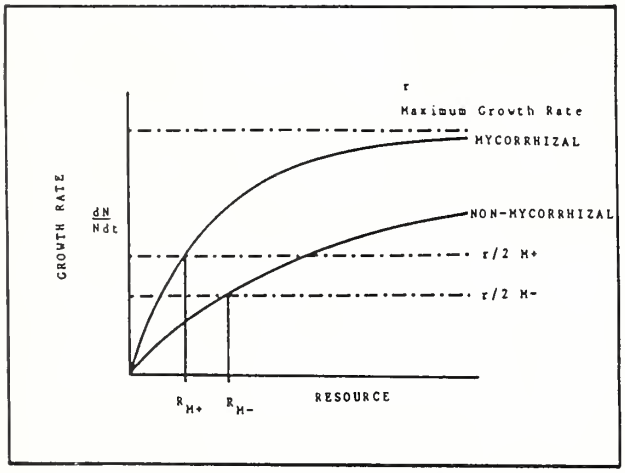

Fig. 5. Hypothetical growth rates of a mycorrhizal and a non-mycorrhizal example of the same species growing at different phosphorus levels (Resource). For the mycorrhizal species to grow at one-half its maximum growth rate (r/2 M+) the species will require an available level of the resource at R_{M+}.

When considering two different mycorrhizal species (species A and B), Fig. 6, there may be a difference in growth rate at a given soil phosphorus level. Although B may be able to overgrow A at a high phosphorus level, A will better compete than B at lower available phosphate levels (R_A).

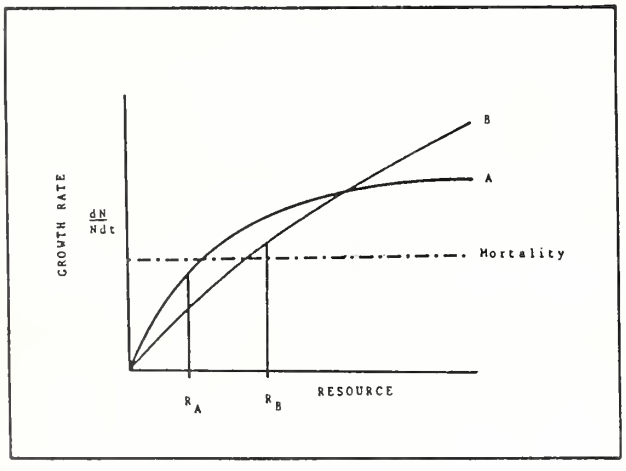

Fig. 6. Hypothetical growth rates of two different species, species A and B, at various phosphorus (Resource) levels. The maximum potential growth rate of B exceeds that of A.

When both plants are growing in the same soil or at the same phosphorus level, available phosphorus decreases. The dynamics of such a population may be predicted by knowing the relative mycorrhizal dependency of the individual species and the relative efficiency of the VAM fungi present, Fig. 7. In this situation, as phosphorus is depleated to a level, R_A, at which species B is no longer able to compete, then species A increases and excludes B. In this scenario, there is a gradual competitive exclusion of species B by species A as available phosphate decreases.

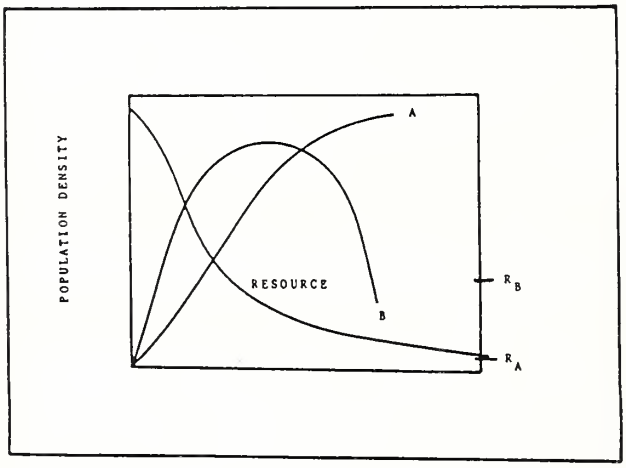

Fig. 7. Hypothetical population dynamics of two species, A and B, as an essential resource is depleted. Compare with Fig. 6.

Because the dynamics of nutrient availability change both temporally (seasonally and with succession) and spacially, the VAM fungi must be adapted to a wide range of hosts and edaphic factors. One area of mycorrhizal research that needs careful and extensive investigation is that of specificity of VAM strains or species for hosts. One may postulate that during succession there will be a selection pressure for those VAM species best adapted to nutrient uptake. However, in most ecosystems, reports indicate that there are a variety of VAM species present. The presence of many species and genera of VAM fungi may represent differences in microniches in plant communities or may be a function of special adaptations of selected VAM fungi to individual plant species.

When competition in a nutritionally heterogeneous environment is considered, one of the predictions of Tilman (1982) is that species richness rises rapidly with enrichment, reaches a peak in moderately resource-poor habitats, and then declines. Using selected soil and vegetation data from semiarid plant communities in western Colorado we find that this theory is applicable, Fig. 8. Those species that occupy the most nutritionally deprived habitats include plants that are known to be obligately mycorrhizal species (the pines and junipers), and they exhibit stress-tolerant strategies (Grime, 1977). Under nutrient stress conditions the actual net cost of symbiosis in such plants may be negative when compared to the "benefits" of growth enchancement.

Thus there appears to be a convergence of ideas, theories, and data regarding the interactions of secondary succession, mycorrhizal dependency in plants, resource competition, and survival of mycorrhizal fungi. Most plants live in relatively heterogeneous environments in terms of nutrient

availability. In general, survival of VAM fungi is closely tied to the survival of the appropriate host plants. Plant survival is tied to available nutrient levels which are in constant flux and which may be altered as a function of the mycorrhizal condition. As nutrient levels decrease or are altered with succession, competitive exclusion of selected species will occur. The final structure of a plant community is a reflection of the strong selection pressure for the essential symbionts, including the mycorrhizal fungi, that constitute the belowground ecosystem.

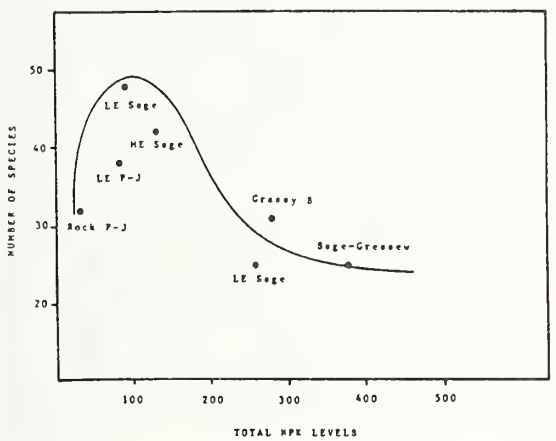

Fig. 8. Species richness (number of species present) ploted against resource availability (total nitrogen+phosphorus+potassium) in a series of semiarid plant communities from western Colorado.

In summary, the long-term survival of mycorrhizal fungi is closely correlated with the survival of the appropriate host plant. The potential survival of a given host is highly dependent on the level of Stress and Disturbance in the environment. Stress may be expressed as a function of available nutrient and water levels. Mycorrhizal fungi enhance nutrient uptake, and there is a gradual decrease in available nutrients with succession. Zero Net Growth Isocline graphics may explain the benefits conferred by mycorrhizal fungi and why there is a selection pressure for the presence of mycorrhizal fungi during succession. The "benefits" conferred upon the hosts by the fungi are the key to their survival.

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MYCORRHIZAL FUNGI AND NUTRIENT MOBILIZATION

By

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Mycorrhizal symbiosis with higher plants has been known for almost 100 years (Frank 1885). Classic work by Hatch (1937) documented the importance of mycorrhizae for mineral nutrition of trees. Ectomycorrhizal fungi are, in return, substantially dependent upon the host plant for carbohydrate supply (Bjorkman 1942). Ectomycorrhizae also increase the effective root surface area for absorption of water and nutrients by partitioning the root carbohydrate supply among fungal hyphae rather than to root hairs (Bowen 1973, Harley 1975).

Fungi have evolved two major symbioses with photosynthetic plants, first as lichens and later as mycorrhizae. In both cases, fungal metabolism products can facilitate solubilization of P and cations through soil weathering. The discovery of lichen production of oxalic acid as a weathering agent (Salter 1856) was followed by the later discoveries of widespread production of oxalate by many fungal taxa (De Bary 1887, Foster 1949), including fungi which are ectomycorrhizal (Graustein 1977, Cromack et al. 1979). An advantage of oxalic acid is that it is energetically inexpensive to produce compared to other Krebs cycle acids, having only 8.5% the caloric value of glucose on a unit weight basis. However, hydroxy siderophores may be produced by ectomycorrhizal fungi (Szaniszlo et al. 1981, Perry et al. 1984) in cases where selective chelation and uptake of Fe is essential to meet physiological requirements. Siderophores are metabolically more expensive to produce than an organic acid such as oxalic acid, but have been hypothesized to make Fe less available to competing rhizosphere microflora, including pathogens (Powell et al. 1982). These fungi, including ectomycorrhizal fungi, have evolved lower cost and versatile biochemical mechanisms such as oxalic acid secretion, which can form complexes with a number of cations as well as increase P availability, and also have evolved highly specific chelating agents such as hydroxy siderophores for Fe.

Recent work on fine roots of Douglas-fir and other conifers (Marshall and Waring 1985) suggests that these roots are endowed with a finite supply of stored carbohydrate as starch at the time of root growth. This would place a premium on efficient use of energy per unit surface area and combine elements of both the Bjorkman (1942) carbohydrate hypothesis and the increased effective surface area hypothesis (Bowen 1973, Harley 1975, 1978). Other intriguing questions are raised if root turnover is being regulated by depletion of original stored carbohydrate supply. Is interconnection among several fine roots essential if a fungal species is to have sufficient longevity to permit sporocarp formation? If so, would not fungal colony longevity exceed average fine root longevity? In the case of fungal mat type colonization by some ectomycorrhizae, mat longevity is sufficient to permit formation of extensive rhizomorph networks (Cromack et al. 1979, Dell and Malajczuk 1985). In Finland, fungal mats may have existed for more than a century in relatively slow-growing northern temperate forests (Hintikka and Naykki 1967). Studies of sporocarp phenology (Fogel 1976, Harley 1971) suggest that fungal sporocarp formation for individual species may vary from more than once per year to once every several years. If fine root turnover is less than a year even in some temperate climates, such as Marshall and Waring's (1984) data suggest, then root networking would be an essential feature of ectomycorrhizal colonization. Fungal networking among roots would be an important feature promoting colony enlargement among multiple fine roots of the same plant; colony enlargement could include adjacent plants of the same species or even different host plant species. In the case of two competing plants, one dominant and one suppressed, there could be partial colony dieback when a suppressed plant dies, while still maintaining the fungal species' presence. The type of ectomycorrhizal formation leading to extensive rhizomorph formation has been shown to be beneficial to eucalyptus nursery trees (Dell and Malajczuk 1984, N. Malajczuk - pers. commun.), especially ones that are past the seedling stage.

Whether ectomycorrhizal fungi can decompose complex carbon substrates remains a controversial question (Lindeberg and Lindeberg 1977). Organic and inorganic substrates cohabit with decomposers, assimilate nutrients in competition with decomposers and in some cases regulate rate of forest floor turnover (Gadgil and Gadgil 1975). Can ectomycorrhizal fungi decompose structural components containing compounds such as cellulose, chitin, pectin, lignin, etc? This question is partly important in facilitating understanding of initial colonization of host root tissue, and partly important with regard to the question of ectomycorrhizal fungi as deocomposers. Lindeberg and Lindeberg (1977) did not find evidence of pectinase activity in several ectomycorrhizal species examined by them, though a closely related decomposer strain of Boletus subtomentosus could do so. A. L. Todd (pers. commun.), using ^{14}C labelled materials, did obtain some evidence of mycorrhizal degredation of complex carbon substrates. Other evidence has shown it possible that some ectomycorrhizal fungi may be decomposers (Linkins and Antibus 1982). If some ectomycorrhizal fungi are decomposers, then their ability to access energy resources becomes greater and more diverse.

A question related to ectomycorrhizal fungi as decomposers is this ability to access essential nutrients such as N and P from organic substrates. This may range from the ability to take up simple compounds such a amino acids for

N and phytate for P, and assimilate them or accessing these organic nitrate forms through enzymatic or other chemical mechanisms from more complex substrates. Some of the early work of Melin (1923) for example, was concerned with the question of mycorrhizal uptake of N in organic substrates such as humus. In acid humus substrates, including decaying wood, sufficient N would not be available as NO_4-N to sustain adequate plant growth. Work by Mork (1927) had shown mycorrhizal colonization of organic substates such as decaying logs. Melin (1923) evidently wanted to understand how N became available for plant growth in such substrates as humus and decaying wood. More recent work on N uptake by mycorrhizal fungi has been pursued by Lundberg (1970) and Lindeberg (1981). Effective fungal colonization with a high degree of surface area to access relatively available N from the exchange complex or from decomposition would be one answer. Such questions lead ultimately to work of Lindeberg and Lindeberg (1977) and others, concerning ability of ectomycorrhizal fungi to be decomposers. The most recent work by Li and Castellano (1984) may help supply a new answer to such questions, in that there exist N fixing bacteria associated with some ectomycorrhizal fungal species colonizing both soil and decaying wood. Work by Ho (1984) may provide increased understanding of P uptake through better knowledge of phosphatase activity.

Work by Rygiewicz et al. (1985 a,b) indicates a potential for mycorrhizal diversity and flexibility in uptake of N. Both NH_4-N and NO_3-N may be utilized, some preferentially by different plant and/or fungus species. For example, some species would have nitrate reductase (Ho and Trappe 1980). Other mycorrhizal species may assimilate N from organic sources; labeled ^{15}N sources will be utilized by C. Bledsoe (pers. commun.) in future experimental work.

Acknowledgments

I wish to acknowledge the fine contribution to my discussion session at the Mycorrhizal Conference by C. Bledsoe, G. Bowen, J. Marshall, N. Malajczuk and D. Perry.

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MICROBIAL INTERACTIONS IN THE MYCORRHIZOSPHERE

By

R. G. Linderman

Introduction

Mycorrhizal effectiveness depends on the optimal interaction of host, symbiont, and environmental factors. One of the least understood, but very important, environmental components is the soil; and the most critical portion of the soil in relation to plant growth is that soil surrounding and influenced by the root called rhizosphere soil.

The nature and importance of the rhizosphere was elegantly reviewed some 20 years ago by Katznelson (1965). He pointed out that the establishment and maintenance of the rhizosphere microbial populations is a dynamic process affected by (a) age and kind of plant, (b) nature and treatment of soil, (c) environmental factors, (d) foliar applications, and (e) interaction of microorganisms. Included in the aspect of microbial interactions, of course, would be pathogenic organisms affecting root health, and beneficial organisms including mycorrhizal fungi. It is important to be mindful that the selective or preferential development of rhizosphere populations occurs in the early stages of plant growth and root development due to root exudation, but thereafter is altered and maintained at some microbial equilibrium by both microbial and plant activity, with both being influenced by soil edaphic and other environmental factors.

From within the complex of rhizosphere organisms are mycorrhizal fungus propagules which respond to root and/or microbial stimuli in the rhizosphere and subsequently form a mycorrhizal association with the host plant. This symbiotic association results in chemical and physical changes in the rhizosphere due to altered host physiology as well as the physical and chemical presence of mycorrhizal fungus hyphae in the rhizosphere and beyond. The mycosphere (Gilbert and Linderman, 1979) selective pressure placed on the residual rhizospheric microflora was considered to be profound and permanent by Rambelli (1973) who coined the term "mycorrhizosphere" to describe the microbial ambient around mycorrhizae.

In our discussions, we hope to develop a better appreciation for the extraordinary microbial complexity of the mycorrhizosphere, provide some overall interpretations of published work on microbial interactions in the mycorrhizosphere, and point out and discuss some experimental problems, with some suggested solutions, that arise in studying these complex relationships.

Microbial Interactions

Microbial interactions in the mycorrhizosphere can be either direct interactions in the soil due to metabolic exchanges between organisms, or indirect interactions mediated by the host plant, via root exudates or within the host plant itself. Most studies and reviews of microbial interactions with mycorrhizae (Dehne, 1982; Hayman, 1978; Hussey and Roncadori, 1982; Schenck, 1981; Schenck and Kellam, 1978) have focused largely on indirect interactions mediated by the host plant. Relatively few studies have examined the direct primary or secondary interactions in the rhizosphere. Not surprisingly, therefore, most authors conclude that the primary effects of interactions are the result of altered host physiology due to the mycorrhizal symbiosis. That changes in phosphorus nutrition can alter membrane permeability and thus root exudation (Ratnayake et al., 1978) does suggest that mycorrhiza formation will lead to a changed exudation pattern that will result in a new microbial equilibrium. However, few studies have actually examined specific microbial responses to that change. Some of those responses could be by organisms that are capable of producing metabolites that could enhance or inhibit plant growth, and thus contribute to the net plant growth response.

Mycorrhiza-pathogen interactions. Pathogenic fungi, bacteria, and nematodes could directly interact with mycorrhizal fungi in the mycorrhizosphere, but this aspect largely has not been explored. Rather, authors or reviews suggest that most of the interaction occurs via altered host physiology where the measured response is a disease incidence or severity index. I am aware of no published study where the behavior of the pathogen has actually been monitored in rhizosphere soil from mycorrhizal vs. non-mycorrhizal plants. Most soilborne pathogen-mycorrhiza (indirect) studies have been done with pathogenic fungi or nematodes; none have been done with bacterial pathogens. As Dehne (1982) pointed out, mycorrhiza formation may also be reduced in roots already infected by root pathogens.

Most of the pathogen-mycorrhiza studies have not taken into account the time variable. Most root pathogens are able to infect roots much more rapidly than any mycorrhizal fungi. So if both pathogen and mycorrhizal fungus are inoculated at the same time, pathogens will generally infect first (Stewart and Pfleger, 1977). Furthermore, many root pathogens (such as Pythium and Phytophthora) have the capacity to rapidly multiply and spread in and on the root so that many new infections can be initiated on other roots under pathogen-favorable conditions. Once mycorrhizae have formed, however, it is conceivable that they could deter root pathogen ingress or development later in the growth period.

The P variable has been considered in some studies where P-amended controls were compared to mycorrhizal fungus-inoculated plants (Baath and Hayman, 1983; Davis and Menge, 1980; Davis and Menge, 1981; Davis et al., 1979), but in most cases P nutritional levels were not comparable between treatments, so conclusions drawn could have been misleading.

In the case of ectomycorrhizae, the mechanisms suggested (Marx, 1972; Zak, 1964) whereby roots are protected from pathogens are generally thought to be that ectomycorrhizal fungi (a) use surplus carbohydrates in the root so that root pathogens are deprived, (b) provide a physical barrier to penetration in the form of the mantle, (c) secrete antibiotic substances inhibitory to pathogens, (d) contribute to a protective microbial rhizosphere population, and (e) induce chemical changes in the host cortical cells that inhibit pathogen ingress and spread. Marx (1972) reviewed the antibiotic production concept, but little if any work has been done to confirm the involvement of antibiotics in situ at the soil-mycorrhiza interface.

Similarly, the physical protection by the fungal mantle has not been further explored beyond the convincing work of Marx and co-workers (Marx, 1972). The production of antimicrobial substances by ectomycorrhizal fungi and ectomycorrhizae has also not received much attention in recent years. Krupa and Fries (1971) identified volatile terpenes and sesquiterpenes extracted in higher concentration from mycorrhizal than non-mycorrhizal roots, and Krupa and Nylund (1972) demonstrated that those compounds were inhibitory to the root pathogens Phytophthora cinnamomi and Fomes annosus in vitro, but no one has demonstrated that those materials actually function in vivo to deter root pathogens. More recently, however, Sylvia and Sinclair (1983) demonstrated that phenolic compounds that accumulate in root cortical cells when ectomycorrhizae form (Malajczuk et al., 1984) increased the root resistance to Fusarium and Pythium invasion.

That ectomycorrhizae can select and maintain a rhizosphere microflora that could protect plant roots from pathogens was suggested by the early work of Katznelson et al. (1962) and later by Neal et al. (1968), but Malajczuk (1979) first actually demonstrated that the ectomycorrhizosphere microflora suppressed P. cinnamomi root rot. Exactly how suppression was accomplished is not totally understood, however.

Considerable effort has been expended studying mycorrhiza-nematode interactions, largely with VA mycorrhizae on agricultural crops. Most have been indirect, host mediated studies wherein changes in nematode behavior within host tissue were described. What, if any, extra-root interactions with associative mycorrhizosphere microbes may occur is unknown. The consensus of opinion (Roncadori and Hussey, 1982) is that altered host nutrition or physiology accounts for observed changes in nematode behavior. Furthermore, most changes are attributed to improved P nutrition. K. Cooper (personal communication) on the other hand has shown altered nematode behavior in mycorrhizal plants regardless of the level of P fertilization suggesting physiological changes independent of nutrition.

More direct observations of pathogen behavior in rhizosphere soil or on the rhizoplane of mycorrhizal vs. non-mycorrhizal plants need to be made. Such have not been made with VA mycorrhizae, but have been made with ectomycorrhizae; some perspectives on ectomycorrhizal-pathogen interactions will be discussed by N. Malajczuk in later discussions.

Symbiotic N_2 fixers. Nitrogen fixation can occur in the soil by free living, root associative, or nodule-forming microorganisms. The general consensus, as will be discussed in more detail by S. Rose later on, is that these organisms are more efficient N_2 fixers if associated with mycorrhizal plants compared to non-mycorrhizal plants. Similar enhanced N_2 fixation can be accomplished by addition of P fertilizer. Again, the consensus opinion is that most of the effect is the result of indirect, host mediated changes rather than a direct effect of mycorrhizal fungi on the N_2-fixing organism in the nodules or in the rhizosphere soil. But as mentioned before, relatively few studies have monitored behavior of the N_2 fixing organisms in soil. Recent exceptions, however, that demonstrate the value of the approach are studies by Bagyaraj and Menge (1978) with added Azotobacter, by Manjunath et al. (1981) with Beijerinckia, and by Barea et al. (1983) with Azospirillum. In these studies plant growth response was primarily measured as the response to dual inoculations. Bagyaraj and Menge (1978), however, also measured rhizosphere population shifts. In all cases synergistic effects between the N_2 fixing bacteria, or added N, and VA mycorrhizae were observed. More studies are needed of this type supporting the concept of the practicality of dual inoculations in agriculture.

Soil fauna. Except for studies on the interaction between mycorrhizae and nematodes, very little attention has been paid to the rest of the soil fauna. That some soil animals feed on fungi is documented, but only the study by Warnock et al. (1982) showed that soil insects (springtails) grazed on the external hyphae of VA mycorrhizae, rendering them ineffective. I am aware of no studies of protozoans feeding on mycorrhizal fungus hyphae, although mycophagous amoebae are known to feed on other fungi.

Functional group selection. The early discussions of the rhizosphere (Katznelson, 1965) and later the mycorrhizosphere (Rambelli, 1973) indicated that a selective pressure is placed on the soil microflora by the root at first, and then by the root and associated microflora. The microbial complexity of the mycorrhizosphere is difficult to analyze. Generally no quantitative differences have been detected between mycorrhizal and non-mycorrhizal roots, but qualitative changes have been noted. When those changes involve N_2 fixing organisms or phosphate solubilizing organisms, the implications for plant growth are apparent. Most of the other functional groups that may affect plant growth or fungal activity, however, have not been examined. For example, a number of functional groups might be expected to affect plant growth if they were increased in the mycorrhizosphere. In addition to nitrogen fixers and P solubilizers, plant growth could be affected by siderophore producers, plant hormone producers, vitamin producers, exopathogens, or even other competitive fungi such as root pathogens. Similarly, fungal activity (mycorrhizal fungi themselves and/or fungal pathogens) may be affected by secondary interactions with functional groups of organisms such as chitinase producers, siderophore producers, antibiotic producers, pathogen suppressors, facultative anaerobes (possibly N_2 fixers and/or ethylene producers), or hyperparasites. The key to the analysis of functional groups is to use some selective medium that assays for specific function (i.e., a chitin-base medium to assay chitinase producers) or a bioassay that measures some biological function. Such assays may indicate a net change in function when comparison is made between mycorrhizal and non-mycorrhizal plants. With this strategy, activity, not taxonomic groups, is assayed, preferably over a time course. We have used this approach to analyze VA mycorrhizosphere changes, and later in our discussion J. Meyer will discuss the subject in greater depth.

Experimental Problems, Approaches, Recommendations

There are significant problems encountered when designing experiments and interpreting results

concerning microbial interactions in the mycorrhizosphere. If we can correct some of the experimental design problems, presumably we could better interpret results.

A major problem in interpreting microbial interaction results comes from having all soil variables superimposed in the test system. Thus the experimental system precludes determination of whether effects are the result of direct microbial interactions or indirect, host-mediated interactions. A useful, but underutilized, approach to separate variables is to use the 'split root' technique such as was used by Menge et al. (1978) to demonstrate that tissue P, not soil P directly, was responsible for VA mycorrhizal inhibition. Similarly in principle, Sanders (1975) applied P through foliar feeding to accomplish separation of the mycorrhizal fungus from the source of P. The split-root technique could be extremely useful in studying or determining direct vs. indirect microbial interactions simply by comparing plant response to two organisms on the same side of the split or on opposite sides.

Rhizosphere studies have long been plagued by variation resulting from different sampling procedures and what one calls that which has been sampled. Careful, detailed explanation of how rhizosphere soil was collected is essential. Furthermore, experiments on the mycorrhizosphere should also take into account the special influence of the external hyphae of the mycorrhizae that may extend the dimensions of the mycorrhizosphere. With ectomycorrhizae, the question could be raised as to whether there is really a rhizoplane as we define it for non-ectomycorrhizal hosts.

Another fundamental problem is how one goes about detecting and measuring significant microbial responses in the mycorrhizosphere compared to the non-mycorrhizal rhizosphere. This question appears to have been one of the most significant obstacles to progress in rhizosphere biology in general. Measuring quantitative shifts has not been meaningful in most cases, but examining the mycorrhizosphere for qualitative shifts appears to be the right approach. Two strategies seem to be useful to monitor qualitative shifts. One is to monitor drug-marked strains of the organism in question (representing some functional group), i.e., Rhizobium, Pseudomonas, Bacillus. Antibiotic resistance markers are not difficult to obtain or develop, and this method allows one to add the organism and monitor its development or survival on a selective medium containing that antibiotic. A second approach is to assay shifts in functional groups on selective media or with specific bioassays. For example, ethylene producers can be quantitatively compared in mycorrhizosphere soil vs. rhizosphere soil from non-mycorrhizal plants by measuring the total amount of ethylene generated per soil volume per unit time. If significant differences occur, further definition of the responsible organisms could be pursued. This approach would allow major effects to be detected without getting bogged down in detailed analysis of organisms that may not have any net effect on plant growth.

Comparison of results from one study to the next is often impossible because cultural conditions are rarely standardized. It is desirable in the experimental system to attempt to compare rhizosphere soil from mycorrhizal and non-mycorrhiz l plants with all conditions being equal except for the presence of mycorrhizae. Major effort should be made to equalize the P level in plant tissues. In some cases results might be most meaningful if several P levels are compared or, if N_2-fixing organisms are involved, several nitrogen levels.

Time is probably the most overlooked variable in microbial interaction studies. For example, rhizosphere populations equilibrate over time as a dynamic process. Age of plant roots changes, seasonal changes occur, stage of mycorrhizal development changes with time, and if the microbial status is monitored at only one arbitrary point, this dynamic phenomenon will not be adequately or accurately described. Of course building multiple harvests into the experimental plan increases the labor-intensity of the study, often beyond a reasonable limit. Nevertheless, the short-fall of single harvest experiments should not be overlooked.

Genetic variability of host plants as well as mycorrhizal fungus strain differences should be given more attention than at present. Different strains or species of fungi will induce different kinds and degree of physiological change in the host tissues, i.e., different membrane permeability and thus different root exudation patterns. While studies with a single host and/or a single strain of mycorrhizal fungus may be quite valid, trends should be confirmed by comparative studies with different hosts and/or different fungi.

Microbial interactions in the mycorrhizosphere are complex and accordingly need special approaches to sort out key activities. Understanding that the establishment and maintenance of a microbial equilibrium is a dynamic, ever-changing process throughout the life of the plant makes us even more aware of the need to develop tools to describe the process, and ultimately to better manage it to improve plant growth and development.

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ANALYZING THE VA MYCORRHIZOSPHERE

By

Julie R. Meyer

In recent studies, we assayed groups of naturally-occurring saprophytic microoganisms associated with VA-mycorrhizal (VAM) or nonmycorrhizal roots to detect possible changes in microbial populations due to the establishment of VAM. Using qualitative assays which would reflect a specific function of the microbial group, we observed both increases and decreases in functional groups as well as taxonomic groups of microorganisms in the rhizosphere of mycorrhizae compared to non-mycorrhizal roots.

The significance of these results is that a change in a functional group, more than a change in a single species, reflects a net change in the rhizosphere microbial equilibrium. A functional group can consist of one or many species which have a similar effect on the environment, such as fixing N_2 or using a certain substrate. Thus it seems that this approach may be particularly useful to study how the rhizosphere environment is affected by VAM establishment. Since we are most interested in microbial processes influencing plant growth, we can focus on how the presence or absence of VAM affect populations or activities of microorganisms known to affect plant growth or welfare.

One problematic aspect of rhizosphere studies is sampling procedure. Where does the rhizosphere end and the root surface, or rhizoplane, begin? Sampling procedure will influence the results obtained in rhizosphere studies, especially when comparing two treatments such as mycorrhizal and non-mycorrhizal roots. Different microbial populations were isolated in our studies from the rhizosphere soil samples, obtained by gently shaking adhering soil into dilutent, than from the rhizoplane samples, which included the washed, macerated roots. This demonstrates a spacial distribution of the rhizosphere populations. For example, greater numbers of total bacteria and specifically pseudomonads were isolated from rhizoplane samples of VAM, but no differences, or reductions in populations were observed in the rhizosphere samples from the same plants.

Qualitative assays allowed detection of microbial changes even when the total number of rhizosphere microorganisms seemed the same. For example, we did not see changes in numbers of bacteria or actinomycetes isolated on general media from rhizosphere soil after VAM establishment, but populations of specific groups did change. More bacteria were isolated on low N medium under anaerobic incubation, conditions which would favor N_2-fixing bacteria, from the mycorrhizosphere than from rhizosphere soil of non-mycorrhizal roots. We did not test the N_2-fixing capability of these isolates. We feel this topic deserves further study and should be expanded to consider other microbial processes involved in nutrient availability to plant and fungus, such as microbial transformation of organic matter, transformation of amino acids to NH_4^+, and mineralization of P, Mn, Fe and other microelements.

Workers in the field of biological control of plant pathogens have identified several functional groups of microorganisms which directly affect pathogens by, for example, degrading fungal cell walls, producing antibiotics, or competing for a specific nutrient or growth factor (Cook and Baker, 1983). We included several of these groups in our VAM studies and observed that numbers of Streptomyces spp. (for example, actinomycetes with known antibiotic production) and of chitinase-producing microorganisms were smaller in the mycorrhizosphere. Evidently, microbial changes which can affect plant pathogens may occur during VAM establishment. Qualitative assays of particular microorganisms may be useful in VAM/pathogen interaction studies performed under conditions in which pathogen, symbiont and the saprophytic microflora are all in place.

The reproduction of the soilborne fungal pathogen, Phytophthora cinnamomi, is very dependent on the microbial activity of the soil. Sporangium production by this fungus is induced by some soil bacteria and inhibited by others. We used P. cinnamomi in a bioassay to test for microbial changes affecting sporangium production in rhizosphere soil after VAM establishment. We observed a reduction in the number of sporangia formed and zoospores released in water extracts from rhizosphere soil from mycorrhizal compared to nonmycorrhizal roots. This type of assay will not reveal any mechanism involved, but will reveal net microbial changes in the rhizosphere which may affect pathogen behavior.

Populations of fluorescent pseudomonads, a diverse group of rhizosphere bacteria, can also be affected by the establishment of VAM. We isolated greater numbers from the rhizoplane of VAM but fewer numbers from the rhizosphere soil. Pseudomonads are very common and include plant growth-promoting types as well as good biocontrol agents and plant pathogens. They are easy to culture and assay, but bioassays are necessary to detect these specific functions. In separate studies, we obtained a siderophore (Fe-chelator)-producing isolate which could increase the growth of inoculated plants in nonsterile soil, presumably by colonizing the rhizosphere so extensively that populations of more deleterious microorganisms, called exopathogens, are diminished (Suslow, 1983). We found that this bacterium would significantly enhance Rhizobium nodulation when introduced on subterranean clover roots. When VAM spores were added to the system, an additive effect on nodulation, root growth and shoot growth was observed. Additive effects or even concerted activities are probably occurring between VA-mycorrhizal fungi and other common rhizosphere microorganisms which influence plant growth under both experimental and natural conditions.

In addition to altered root physiology and root exudation patterns from VAM colonization (Hayman, 1983; Schwab et al., 1983), the role of the fungus itself in influencing rhizosphere microorganisms should not be overlooked. Fungi in soil provide both a surface area and substrate for bacterial and actinomycete growth (Gilbert and Linderman, 1971; Lockwood, 1968). Whether some species are specifically adapted to live in association with fungi is not known and this would be worthy of

study in relation to VA-mycorrhizal hyphae and spores. Such studies have begun with ectomycorrhizal fungi (Li and Castellano, 1984; Richards and Voigt, 1964). The distribution of bacteria and actinomycetes may be influenced by the fungal mycelium which provides a pathway for movement from place to place. Although the plant no doubt exerts the most powerful influence on the microbial equilibrium, there are reasons to consider the influence of the fungal partner as well.

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PERSPECTIVE ON ECTOMYCORRHIZAL-PATHOGEN INTERACTION

By

N. Malajczuk

Introduction

Since Frank coined the term 'mycorrhiza' to describe a fungus-root association, nutrient uptake had been considered as the primary role for these symbiotic relationships. In 1964 Zak suggested a further role for ectomycorrhizas contributing to improved growth, namely protection from invasion by potential pathogens; and a number of mechanisms for this phenomenon were proposed. Subsequent experimental studies by Marx in the early seventies developed this concept further. Since then a number of authoritative reviews and additional supportive research studies have highlighted this ectomycorrhizal role (Marx, 1972; Sinclair et al., 1981).

The concept that ectomycorrhizas can deter plant pathogens represents one of the most outstanding examples of natural biological control resulting in improved tree growth and survival. This protective effect of ectomycorrhizas is generally considered to be a passive phenomenon, secondary to nutrient uptake by roots of higher plants which appear to have co-evolved with fungi to exploit specific environments (Malloch, Pirozynski and Raven, 1980). Alternatively, one may argue that the evolution of plant parasitic fungi concurrently with the domination of higher plants (Pirozynski, 1983) resulted in pressure for tree species to develop root structures to ward off potential pathogens for long term survival. However, irrespective of the evolutionary catalyst which resulted in the formation of ectomycorrhizas, their contribution to tree growth and root longevity are likely to have contributed to the dominance of trees and shrubs in the native vegetation of large parts of the earth's surface.

Attributes of ectomycorrhizas in relation to limitation of disease development.

In considering the mechanisms by which ectomycorrhizas afford protection from disease to feeder roots, there is a need to put in perspective the zones of interaction between the fungal symbiont and pathogen. From the pathogen point of view these would include the interactions (a) external to the root surface, (b) at the root surface, and (c) within the host tissue.

(a) Interactions external to the ectomycorrhizal root surface. It is well documented that ectomycorrhizal fungi produce a significant proportion of their biomass as external hyphae and rhizomorphs radiating considerable distances from the root surface (Bowen, 1973). Their domination of the soil substrate has been shown by Gadgil and Gadgil (1978) to reduce litter breakdown under pines, presumably out-competing decomposing microorganisms. Moreover, Schisler and Linderman (1984) showed a marked reduction in Fusarium species, including root pathogenic species, in forest soils as compared to pastures or nursery soils. Competition and antibiosis are two likely mechanisms by which the symbiotic fungi affect other microorganisms, yet precise investigations are lacking in the literature. Microorganisms which may be associated with the symbiont's hyphae (the hyphasphere populations) and their interrelationship with pathogens have been totally neglected.

(b) Interactions at the root surface. Most of the current information on the protective effect of ectomycorrhizas to pathogens has focused on the root surface, primarily because this is the most obvious site for the interactions to occur. Prevention of infection has been attributed to one or a combination of the following factors: 1. Antibiotic production by the mycorrhizal fungi creating an environment unfavorable for spore germination and mycelium growth; 2. Provision of a physical barrier to the pathogen in the form of a compact fungal mantle; 3. Reduced and altered root exudation patterns affecting chemotaxis of the pathogen spores and/or mycelium; and 4. Stimulation of an antagonistic microflora associated with the mantle.

The first two mechanisms are inherent characteristics of the fungal symbiont. Extensive in vitro studies have been conducted on the production, chemical nature, and root pathogen inhibition effects of antibiotics produced by ectomycorrhizal fungi. There is still much more to be learnt of the significance of antibiosis in the rhizosphere. Undoubtedly, fungal symbionts with this capability would debilitate potential competitors including pathogens. Weakened pathogens would in turn find the mantle difficult to penetrate for root infection to proceed.

Factors 3 and 4 are also interrelated; changed root exudate patterns would not only affect the behavior of the pathogen but would stimulate a rhizosphere population which has been shown to be distinct from that occurring on non-mycorrhizal roots. Malajczuk (1979) reported bacterial populations as high as 10^{10} cells/g dry weight eucalypt root located throughout the mantle both within and between symbiont cells. Many of these microorganisms also exhibited strong antibiotic effects on Phytophthora and Pythium root pathogens. In a recent study of actinomycete populations in soil in Western Australia, Keast and Tonkin (1983) showed that populations associated with Pinus radiata roots were strongly inhibitory to the growth of P. cinnamomi in agar culture, but had no effect on the ectomycorrhizal fungal symbiont Laccaria laccata. Studies of this type are scarce in the literature and would warrant further attention.

Recent developments in electron microscopy have enabled a more precise location of microorganisms in both soil and rhizosphere, and will undoubtedly form the basis for future studies. The scanning electron microscope has the advantage over light microscopy in that it allows for 3D visualization of interactions that occur on the root surface. In our laboratory, we have examined such interactions between the root pathogen, Phytophthora cinnamomi, and eucalypt ectomycorrhiza following point inoculation of these roots with suspensions of motile zoospores (Malajczuk and Sanfelieu, 1984). These studies have visualized the fate of the pathogen's infective propagules, be it either failure to penetrate the ectomycorrhizal mantle and/or being lysed by a range of morphologically distinct bacteria and fungi. Incorporation of tracer dyes and/or metals onto the pathogen propagules, and incorporation of X-ray microanalysis

with the scanning electron microscope, may further enhance the precision of this technique.

(c) Ectomycorrhizal host mediated response to the pathogen. The response of the host to invasion by any pathogen or non-pathogen, whether fungal, bacterial or viral, elicits the production and accumulation of phytotoxins which may inhibit the microorganism (Deverall, 1977). We see a similar response occurring when the fungal symbiont initiates the state of symbiosis and is contained within the epidermal and outer cortical cells of root tissue. Antimicrobial, polyphenolic compounds appear to be associated with root tissue following infection of the fungal symbiont (Malajczuk et al., 1984). In a recent study by Sylvia and Sinclair (1983) a strong correlation was obtained between these phenolic compounds accumulating in mycorrhizal roots and reduced infection by the pathogens Fusarium oxysporum and Pythium irregulare. These 'immunizing' reactions elicited by ectomycorrhizal root invasion undoubtedly raise the level of resistance of roots to invasion of potential pathogens. One of the more immediate questions that awaits attention is the extent of this reaction within the host plant. Does this phenomenon extend to nearby non-mycorrhizal roots? Can it also reduce the activity of foliar pathogens?

Problems and prospects

Investigations to date have focused on isolated ectomycorrhizal root systems, and some of the mechanisms reducing activity of pathogens associated with these roots have been determined. However, there is a complete lack of research which integrates these observed effects and applies them to whole tree systems. To date, limited studies have concentrated on the seedling stage with little or no information available for outplanted seedlings and maturing trees growing in environments where disease problems exist. One would imagine an array of problem areas relating to spatial and temporal factors that need to be considered, particularly in changing root structures and fungal succession associated with a developing tree root system. Answers are needed to such questions as - Do late colonizing fungi provide as effective protection as do early colonizers? How is pathogen infection of non-mycorrhizal roots, even suberized roots some distance from the mycorrhizal root, influenced by the symbiotic relationship? Do mycorrhizal roots compensate by increasing nutrient uptake following loss of feeder roots from pathogen infection?

In the long term there is a need to consider the protective role of ectomycorrhizas as well as their ability to increase nutrient uptake in mycorrhizal inoculation programs if we are serious about maximizing gains in growth and survival in outplanted tree seedlings.

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TRIPARTITE ASSOCIATIONS: A CARBON-NITROGEN-PHOSPHORUS GIVE AND TAKE

By

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The most beneficial contribution of rhizosphere microorganisms to plant development is to supply nutrients essential to growth. As nitrogen and phosphorus are commonly of limited supply in soil, microorganisms associated with nitrogen-fixation and enhanced phosphorus solubilization and uptake are of primary importance.

With the exception of a very few plant species, most vascular plants are mycorrhizal. Without doubt, mycorrhizal fungi, whether by increasing the absorbing surface area of the roots or by releasing enzymes into the soil that solubilize phosphate, enhance phosphorus uptake by the host plant. In contrast to the mycorrhizal condition, only a few plants support nitrogen-fixing microorganisms in a symbiotic relationship: legumes and nodule-occupying Rhizobia; actinomycete-nodulated plants; blue-green algae on the roots of Cycads and the rhizoplane nitrogen-fixing bacteria of grasses. Grasses, legumes, and actinorrhizal plants are also typically mycorrhizal.

The symbiosis of a flowering plant, a mycorrhizal fungus, and a nitrogen-fixing microorganism has been the subject of interest since 1896 when Janse first described such a tripartite association between the legume Pithecolobium montanum, a bacterium, and a fungus. Jones (1924) examined 18 species of nodulated legumes and found that 15 were colonized by vesicular-arbuscular mycorrhizal fungi. Asai (1944) first suggested that mycorrhizae were a necessary precondition for effective nodulation in many legumes. Not until the early 1970's had the influence of mycorrhizae on nitrogen-fixation been investigated. Schenk and Hinson (1973), Crush (1974), and Daft and El Giahmi (1974, 1976) found that the weight of nodules, amount of nodular tissue, nitrogen and phosphorus content, and the rates of acetylene reduction were greater in mycorrhizal nodulated plants than in non-mycorrhizal nodulated plants. Carling et al. (1978) demonstrated that the nitrogen-fixing capability of soybeans increased in response to added increments of phosphorus fertilizer and (or) mycorrhizal infection and suggested that enhanced nodulation and nitrogen fixation is a response to the improved nutrition of the host plant rather than a direct effect on the nitrogen-fixing endophyte.

Increased plant growth and subsequent higher rates of photosynthesis as a result of the mycorrhizal association could explain the increased nodulation and higher rates of nitrogen-fixation if the photosynthates were translocated to the endophyte. Carbon flow to the symbionts and photosynthate partitioning were investigated by Paul and Kucy (1981). They found that the fungus received 1% of the photosynthate and the nodules received from 7-12%, and that more CO_2 was evolved by mycorrhizal beans than the non-mycorrhizal controls. In addition, the fungus used 4% of C^{14} supplied to the host plant; nodules with mycorrhizae used 6% while mycorrhizal nodulated beans used 12% (Kucey and Paul, 1982), suggesting an increase in photosynthesis to compensate for the drain of the carbon compounds by the two endophytes.

Nitrogen fixation in actinorrhizal plants was overlooked by scientists because these plants are not of agricultural importance and, quite the contrary, many were considered a nuisance by foresters until silvicultural benefits could be attributed to them. Over 170 species in 8 families of vascular plants are recognized as nitrogen-fixing components of the ecosystem (Barea and Azcon-Aguilar, 1983; Torrey and Tjepkema, 1983).

Mejstrik and Benecke (1969) found more phosphorus in the leaf tissue of ectotrophic-mycorrhizal Alnus viridis than non-mycorrhizal plants. The endomycorrhizal associates of Alnus, Cercocarpus, and Dryas have been reported by Williams and Aldon (1976). In addition, 25 species of actinorrhizal plants from 7 families were either ecto- or endomycorrhizal (Rose, 1980). In a greenhouse experiment designed after the landmark experiments with legumes, Rose and Youngberg (1981) found increased growth, higher levels of P and N, larger nodules, and more nitrogen-fixation in tripartite Ceanothus as compared to nodulated-only plants. In a similar study with Hippophae in sand dunes in the British Isles, Gardner et al. (1984) reported increased P content, elevated carbohydrate translocation, more growth, and greater nodule activity with mycorrhizal plants.

However straight forward the results and conclusions from the above studies appear, the interpretations of results from research with nitrogen-fixing plants and with mycorrhizae and plant nutrition are prone to error due to problems with current technology and the misapplication of methods. To quote Harley, "Wherever there is a reasonable doubt about the validity of a method or of a question, it is important to try to resolve that doubt before embarking on a process of field sampling with all the labor that this entails" (Harley, 1971). Errors are compounded when studying tripartite systems - biases with N-fixing measurements and assumptions concerning mycorrhizal determinations enter into any calculation. The literature is plagued with conflicting reports - over estimations, under estimations, mis-identifications.

Conard et al. (1983) summarizes the techniques for determining N fixation and includes the limitations of each method: 1. Acetylene reduction - assumption of 0.3 mole N/1 mole acetylene may not be applicable to each situation. Assessment of nodule biomass, especially in the field, may be impossible. 2. Accretion (chronosequence) - long term studies with presumptions of similar initial base-line N over sites and age sequences. Also, measures change over time, not a rate of fixation. 3. Natural isotope - the ratio of N^{15}/N^{14} is different in the soil and the atmosphere. It is assumed that the nitrogen fixed in nodules will more closely resemble the atmosphere. Large within-pool variation makes studies over any large area difficult to correlate. 4. N^{15} radio isotope - measure the atom % excess of N^{15}. This allows a direct comparison, but it is expensive and has a high within-pool

variation. 5. Xylem sap - N compounds fixed in nodules are transported in specific compounds in the xylem. By determining the rate of flow, one is able to calculate the concentration. It will be a valuable technique for deep-rooted plants, but has only been worked out for legumes and alder.

Measuring the amount of mycorrhizal colonization is a destructive and difficult task. Lack of standardization has led to conclusions that cannot be related to the results of other researchers. Each technique has its own biases and limitations (Giovannetti and Mosse, 1980): 1. Intersect points - Gridline methods, are 2-dimensional and may not see the structures on the "underside". 2. +/- fungal structures in a microscope field - has a high standard of error. 3. +/- fungus in a standard root length - high error factor, other than fungus may stain. 4. Visual estimate (including colorimetric) - subjective, to nearest 10% only. Not all fungal roots show color. 5. Morphometric (Toth and Toth, 1982) - squashed root and dot-intersect method; difficult to discern fungal structures in squashed mount. 6. Immunofluorescent (Kough et al., 1983) - genus-specific. Laborious.

Elucidating the biology of Frankia and the isolation and degree of infectivity of endophyte strains are the research endeavors for the actinorrhizast (Torrey and Tjepkema, 1984). The research emphasis of mycorrhizasts is as wide as the geographic distribution of the scientists involved: cell physiology, ecology, root recognition, taxonomy. The direction of research on tripartite plants, particularly actinorrhizal plants, is more applied, especially in the under-developed regions. Both in under-developed and developed nations, actinorrhizal research is in the area of forestry and land reclamation: 1. Casuarina is the most important fuel tree in the world (Torrey and Tjepkema, 1983). In Senegal at the Microbiology Laboratory, Dr. Dommergues and co-workers are growing isolates of Frankia and VA mycorrhizal fungi that are drought and saline tolerant. They are involved in an ambitious outplanting project (Diem et al., 1981). 2. In Europe, sand stabilization by actinorrhizal plants is important, with an emphasis on alder and Hippophae. 3. In China, over 1 million ha of sand and deficient land is planted to Casuarina. Research on cloning Casuarina and outplanting with inoculated seedlings. Selection of effective strains of endophytes. 4. Many actinorrhizal plants are important in land reclamation in the semi-arid regions of the U.S. S. Williams and students at the Univ. of Wyoming are isolating and screening isolates of native actinorrhizal plants including Purshia, Cercocarpus, Shepherdia. They are designing outplanting studies to determine survivability. Mycorrhizal fungi and their role in the transfer of nutrients across hyphal bridges is being studied using similar designs that B. Ames used for his research at Colorado State University.

Of the utmost importance is the development of workable models from N^{15} and C^{14} work which will enable the researcher to describe what is going on in the field and to predict on a broader base than what the laboratory and greenhouse experiments allow. The development of models will give the ecologist a tool to design experiments and field studies that will yield data on the role of actinorrhizal plants in the nutrient cycling in an ecosystem.

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ECOSYSTEM RELATIONSHIPS AND MODELING OF MYCORRHIZAE

DIRECT AND INDIRECT METHODS FOR DETERMINING MYCORRHIZAL BIOMASS IN FOREST ECOSYSTEMS

By

K. Vogt

Keywords--fine root biomass, fine root turnover, starch, living bark volume, forest floor nitrogen, litterfall nitrogen, mean residence time forest floor N

Introduction

The importance of including fine roots and mycorrhizal roots in ecosystem level carbon and nutrient cycling studies is generally accepted now. However, due to the time consuming and tedious procedures required to obtain good biomass and production estimates for mycorrhizal roots, few quantitative field studies have been conducted (Alexander and Fairley 1983, Fogel and Hunt 1981, Vogt et al. 1982, 1983). If ecosystems are to be more fully understood, alternative methods must be found to quantify the biomass and turnover of fine roots and mycorrhizal roots. Since direct methods appear to be cost prohibitive the alternative would be to develop an indirect method which still yields relatively good estimates of root biomass and is economically feasible.

Indirect methods of determining mycorrhizal biomass can only be used on sites after prior research has shown that a good relationship exists between mycorrhizal root biomass and the indirect parameter. This in itself necessitates obtaining simultaneous data on mycorrhizal biomass and the parameter that is sensitive to changes in this biomass.

A number of different parameters may be used to predict mycorrhizal root biomass and production. It is important to be able to identify those parameters or combinations of parameters that have the strongest influence on the mycorrhizal association at specific sites. It is important to use parameters that not only show a relationship to the mycorrhizal fungi but are sensitive to annual climatic changes or other perturbations.

Based on current research, three general categories which may contain some parameters that are capable of indirectly estimating fine root and mycorrhizal root biomass are : 1) climate, altitude, 2) nutrients, especially nitrogen, and 3) plant energy reserves. Future research will have to determine which individual or combination of parameters will result in the best estimate of mycorrhizal root biomass for different locations and forest types (i.e. evergreen versus deciduous, broadleaf versus needle-leaved).

The following discussion will review data showing the importance of parameters within the three general categories as future predictors of mycorrhizal root biomass. The following discussion is not based on relationships specifically developed for mycorrhizal roots but for fine roots. However, similar relationships should exist for mycorrhizal roots. Caution should be used in applying these specific regressions randomly across many forest types. It appears that the importance of specific factors in having the dominant influence on roots varies as a function of tree life-form (broad-leaved, needle-leaved) and behavior (deciduous, evergreen).

The regressions developed for fine roots can be used in conjunction with site specific estimates of the number of mycorrhizal root tips per gram of root tissue to eventually estimate mycorrhizal biomass. That is, it will be necessary to determine the degree of mycorrhizal infection of a root tip (the proportion of fungal to root material on a root tip) and the proportion of root tips infected by mycorrhizal fungi.

Discussion

The relationships shown in the first two categories were obtained from a world-wide synthesis of fine root data. The results of manipulating this data to examine the relationships between roots, climatic factors and nutrients will be shown here (Vogt et al. 1985).

Climate, altitude. We are able to account for 61% of the variation in estimating fine root biomass using maximum monthly mean temperature for needle-leaved forests (Vogt et al. 1985). The lower the maximum monthly mean temperature, the higher the fine root biomass maintained ($Y = 15{,}475 - 496\ X$, where Y=fine root biomass in kg/ha and X=maximum mean monthly temperature in C). Maximum monthly mean temperature should be combined with some other parameter to improve the prediction of fine root biomass. This relationship between temperature and fine root biomass did not occur for broad-leaved forests for which root data was available (Vogt et al. 1985).

For needle-leaved forests, altitude was strongly related to the amount of fine root biomass maintained: the higher the altitude the greater the fine root biomass maintained (Vogt et al. 1985). Seventy-eight percent of the variation in fine root biomass was explained by altitude in needle-leaved forests ($Y = -120 + 9.05\ X$ where Y=fine root biomass in kg/ha and X=altitude in meters). No relationship was apparent between fine roots and altitude for broad-leaved forests.

Nitrogen. Fine root biomass and turnover cannot be predicted from the same nitrogen containing components within an ecosystem. Root turnover may be predicted from the amount of nitrogen added annually in aboveground litterfall while root biomass had no relationship with litterfall nitrogen. In broad-leaved ($\log Y = 4.59 - 0.025\ X$ where Y=root turnover in kg/ha/yr and X=litterfall nitrogen in kg/ha/yr) and needle-leaved forests ($\log Y = 4.38 - 0.020\ X$ where Y=root turnover in kg/ha/yr and X=litterfall nitrogen in kg/ha/yr), 86% and 72% respectively of the variation in root turnover was explained by litterfall nitrogen (Vogt et al. 1985). In needle-leaved forests, 72% ($Y = 827 + 6.22\ X$ where Y=root turnover in kg/ha/yr and X=forest floor nitrogen content in kg/ha) of the variation

in root biomass was explained by the nitrogen content of the forest floor while only 31% of the variation in root turnover was explained by forest floor nitrogen content. If the mean residence time of nitrogen (MRTN) in the forest floor was regressed against root biomass or turnover for needle-leaved forests, 86% of the variation in root biomass and 56% of the variation in root turnover was explained by the MRTN in the forest floor (Vogt et al. 1985).

Starch, living bark. For Douglas-fir (Pseudotsuga menziesii (Dougl.) Forbes) stands in the state of Washington, fine root biomass may be predicted from measurements of starch contents of living bark on a stand basis ($Y = -2100 + 7.84 X$ where Y=fine root biomass in kg/ha and X=starch g/ha at breast height) (Vogt et al. 1984). This technique is based on the fact that there is a direct correlation with the starch content of a 1 cm thick band of living bark at breast height per hectare and fine root biomass per hectare ($r^2=0.85$). Root production may be estimated from frequently sampling for starch contents in living bark throughout the year.

If fine root biomass data is available, fine root turnover may be predicted for needle-leaved forests located in cold temperate regions. In the Pacific Northwestern U.S.A., there is a strong relationship between conifer fine root biomass and turnover ($r^2=0.85$) (Vogt et al. 1985). In these ecosystems, annual root turnover is almost equivalent to root biomass. Recently, Marshall (1984) has suggested that patterns of starch deposition and depletion and soil temperature may be used to estimate fine root production and turnover for conifers. Marshall will be presenting a poster on this approach during the 6th NACOM.

It is apparent from the above mentioned relationships that indirect methods have great potential for being used to estimate mycorrhizal root and fine root biomass. Presently these relationships are based on a small data base available for fine roots (Vogt et al. 1985). It will be necessary to expand this data pool to specifically examine the importance of these relationships for mycorrhizal roots in a wide variety of forest types. This would give the capability of selecting those parameters most sensitive to mycorrhizal root biomass and fluctuations in this biomass.

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DIRECT MEASUREMENT OF ECTOMYCORRHIZAL BIOMASS

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Introduction

Accurate estimation of fine root biomass is difficult and time consuming due to physical problems of separating roots from soil, laborious counting of root tips under magnification, and the large sample numbers needed to document significant biomass changes. Estimating the contribution of small roots to net primary production (NPP) has, therefore, sometimes been ignored or arrived at indirectly (Newbould 1967, Whittaker and Likens 1973, Whittaker and Marks 1975). Reiners (1973) acknowledged that carbon contribution by roots is much more difficult to measure than for aerial components. He states, "the simplest assumption is that the ratio of root production to root biomass equals the ratio of shoot production to shoot biomass." This assumption appears elsewhere in the literature and has reinforced the idea that aerial debris fall constitutes the major source for return of nutrients to soil (Gray and Williams 1971, Heilman and Gessel 1963). For example, Gray and Williams (1971) state, "the root:shoot ratio of living plants suggests that, on average, about half as much root material enters the soil as compared with surface litter...." Recent studies in the Pacific Northwest that used a harvest (soil-coring) and direct root observation technique show that assumptions of root production based on shoot production-to-mass ratios seriously underestimate small root mass (Fogel 1983). Data for Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) and Pacific silver fir (Abies amabilis Dougl. ex Forbes) show that fine roots and mycorrhizae contribute a major portion of the organic matter entering decomposition and consititute 60-70% of annual stand net primary production (Fogel 1983, Fogel and Hunt 1983, Grier et al. 1981).

The Need for Accurate Quantification

Despite the widespread occurrence of mycorrhizae and their well-documented importance in nutrient cycling (Harley and Smith 1983, Marks and Kozlowski 1973, Sanders et al. 1975, Smith 1980), they are generally not included in nutrient cycling studies of forest ecosystems (Henderson and Harris 1975, Likens et al. 1977, Reichle 1970, Whittaker et al. 1979). To fully understand ecosystem processes controlling immobilization and mineralization of plant nutrients, the dynamics of fine root turnover must be considered. Data from a 55-year-old Douglas-fir stand in the Coast Range of Oregon (Fogel and Hunt 1979, 1983) show that fine roots ($\leq$5 mm in diameter) and mycorrhizae compose 6% of tree biomass and contribute 50-58% of annual stand organic matter return (throughput) to soil (14.6-18.8 Mg/ha). This is 2 to 5 times more material than is produced by aerial tree components.

With annual mycorrhiza "die-back" equaling 56% of maximum mycorrhiza standing crop in the 55-year-old Douglas-fir stand, implications for nutrient cycling become obvious. Annual release of N, P, and K by fine roots and mycorrhizae in this stand are 113, 26 and 39 kg/ha respectively (Table 1). Roots contribute 43% of total stand N release as do soil hyphae and sporocarps combined (Table 2).

Previous regional and global carbon budgets (e.g., Reiners 1973, Rodin and Bazilevich 1967, Whittaker 1962, Whittaker and Likens 1973) based on the assumption that the ratio of root production to mass is 0.2 to 1.0 times shoot systems need reevaluation. The harvest approach suggests that a ratio of 6.5 to 7.4 is realistic for Douglas-fir (Fogel, in press).

Reporting Stand Parameters and Methods

For fine root data to be most useful, detailed reports of stand parameters and methods are needed. Soil type (including organic matter content) and depth, rooting depth, tree density and age, and stand phenological status at the time of sampling are important. Details of field sampling and laboratory root recovery methods are also critical. If a soil corer is used, diameter and volume should be noted. If small diameter cores are used in soils with low rooting densities, replication should be increased. Other important sampling data include time of year, sampling frequency, and depth of soil sampled. Ambiguity in reporting laboratory procedures is a common problem. Because diameter size range and functional classification of fine roots are not standardized, they must be defined in each study. Most studies use a 2- or 5-mm diameter as the upper limit or use a number of size classes: 2-5 mm in diameter, $\leq$2 mm, $\leq$1 mm, etc. (Santantonio et al. 1977). Ectomycorrhizae should be treated as a separate functional class of roots because they are symbiotic structures with morphological and physiological features that have a profound effect on mineral nutrition of trees.

Converting root tip numbers to biomass requires an estimate of mean tip dry weight. Such estimates are rare and consequently many reports of ectomycorrhiza numbers cannot be converted to biomass. Because ectomycorrhizae produced by different host-fungus combinations vary in morphology, and thus in weight, a biomass conversion factor must be determined separately for each stand or tree species. Ectomycorrhizae of Monterey pine (Pinus radiata D. Don), for example, have been reported to weigh 4.3 x 10^{-5}g dry weight per fragment (Marks et al. 1968) and Douglas-fir mycorrhizae 19.7 x 10^{-5}g dry weight per fragment (Fogel and Hunt 1979). Estimates of fine root and ectomycorrhiza biomass resulting from soil coring methods are

difficult to compare given the number of different methods employed to separate roots from cores (Fogel 1983). Current methods involve hand sorting, dry sieving, wet sieving, or combinations of these combined with magnification and air or water separation of organic matter (Fogel and Hunt 1979, Grier et al. 1981, Kimmins and Hawkes 1978, Marks et al. 1968, Persson 1980, Santantonio 1979). Choice of sieve size can greatly affect efficiency of recovering ectomycorrhizae. Many ectomycorrhizae range in size from 0.5 to 3.0 mm long and 0.15 to 0.6 mm in diameter (Fogel 1980). Thus, screens with openings larger than 0.5 mm may permit loss of the smallest roots, a potential problem in several studies (Harvey et al. 1976; McQueen 1968, 1973; Vogt et al. 1980). Fogel (1983) shows that hand sorting is adequate for roots 2-5 mm in diamter, but wet sieving produces a 30-40% increase in biomass estimates for roots $\leq$2 mm in diameter (Table 3). Moir and Bachelard (1969) obtained higher fine root values after wet sieving soil from a Monterey pine stand compared to hand sorting.

Ideally, the proportion of "active" to total ectomycorrhizae should be reported. Root production and loss estimates can be refined by balancing changes in active and inactive roots (Fogel, in press; McClaugherty et al. 1982; Santantonio 1979). Determining the physiological status of large numbers of fine roots has proven difficult, however (Fogel 1983). Clearly, the method of root extraction and physiological state of sampled roots should be given careful consideration and be thoroughly described in the literature.

A Method for Ectomycorrhizal Biomass Estimation in Douglas-fir

Ectomycorrhizal biomass data reported by Fogel and Hunt (1979, 1983) were collected by use of a modified soil core method of Marks et al. (1968). Each month, 9 to 16 cores measuring 5.5 cm in diameter and 11.5 cm long (275 cm^3) were collected in a 55 year-old Douglas-fir stand. Cores were first soaked for 24 hours in 0.1 M sodium pyrophosphate ($Na_4P_2O_7$) to disaggregate clay. Cores were then wet sieved through screens of two mesh sizes, 2.0 mm and 0.355 mm. All material captured on the 0.355-mm screen was transferred to a plastic tray (30 cm long x 20 cm wide x 10 cm deep) to facilitate separation of organic matter by floatation. By use of running water and gentle agitation, all root fragments were returned to the 0.355-mm screen. For processing large numbers of cores, organic matter separation was accomplished by use of soil elutriators (Bel-Art Co., New Jersey[1]). Ectomycorrhizal fragment counts were done on a shallow Plexiglas tray measuring 25 cm on each side. Paper marked with a grid of 100, 1-inch (6.5-cm^2) squares was attached to the bottom. All organic matter recovered from the 0.355-mm screen was suspended in 200 ml of water and uniformly dispersed on the tray. Number of mycorrhizal fragments was recorded for seven randomly selected squares. These values were converted to biomass, then expanded to a hectare basis as described in Fogel and Hunt (1979).

To estimate the relative weights of fungus and host in ectomycorrhizae, we assumed that fungi comprise 40% of ectomycorrhizal dry weight. This value was reported by Harley (1971) and is based on dissection of fungal mantles from beech mycorrhizae. This method underestimates the fungal component because hyphae between cortical cells (Hartig net) are not included. This error could be decreased by measuring the cross sectional area of host and mycobiont and multiplying by an estimate of specific gravity for each component. Other methods for determining the mycobiont portion of ectomycorrhizae are discussed by Fogel (1980).

Conclusions

It is now well documented that fine roots are major contributors to NPP in coniferous ecosystems. Estimates from coniferous stands show 60-73% of total NPP results from roots. Studies in which root turnover has been assumed to contribute half as much biomass as litter to the soil should be reexamined (Persson 1978, Whittaker and Marks 1975). Complete understanding of mechanisms controlling nutrient cycling in forest ecosystems requires accurate assessment of belowground cycling processes. Estimates of fine root biomass from single samples are of little use. Because of large fluxes in root mass over a year, study of the belowground ecosystem requires frequent sampling over several years. To further refine estimates of fine root contribution to nutrient cycling, data are needed on proportions of active to inactive ectomycorrhizae as well as on changes in nutrient levels during cycles of production, senescence, and decomposition of ectomycorrhizae. Currently nothing is know of the degree to which nutrients may be withdrawn back into trees from senescing ectomycorrhizae, and very little is known about the quantity and composition of soluble root exudates and sloughed root fragments. Additional study of fine root dynamics is obviously needed to fully evaluate its importance in nutrient cycling.

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[1]The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the U.S. Department of Agriculture of any product or service to the exclusion of others that may be suitable.

Table 1. Annual release of nitrogen, phosphorus, and potassium by fine roots and mycorrhizae in a 55-year-old Douglas-fir stand, Dinner Creek, Oregon.

Element	kg/ha	% of total element
Nitrogen	113	43
Phosphorus	26	64
Potassium	39	71

Table 2. Annual nitrogen release in a 55-year-old Douglas-fir stand, Dinner Creek, Oregon.

Component	kg/ha	% of total
Total aerial	20	7
Forest floor	19	7
Roots + mycorrhizae	113	43
Hyphae + sporocarps	113	43

Table 3. Increase in root biomass after wet sieving soil cores from a 35-year-old Pinus strobus stand, Ann Arbor, Michigan (from Fogel 1983).

Method	Core	kg roots per ha (% total) 2-5 mm dia.		2 mm dia.[a]	
Hand-sort	1	5,986	(100)	7,378	(69)
	2	2,389	(97)	5,970	(60)
Wet sieve[b]	1	0	(0)	3,351	(31)
	2	74	(3)	4,053	(40)

[a]Includes mycorrhizae.
[b]Method of Fogel and Hunt (1979).

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MEASURING MYCORRHIZAL BIOMASS: PRINCIPLES AND PROBLEMS

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Kewords - Production, mortality, sampling.

Introduction

Reliable estimates of mycorrhizal and fine root biomass are in demand, and no ecosystem or nutrient cycling study is complete without them. Biomass estimates are also central to our attempts to understand what determines the production and mortality of mycorrhizas and how they interact with other components of the soil ecosystem. In this paper I consider a personal selection of some of the problems involved.

Sampling method

Direct assessment of mycorrhizas extracted from soil cores is the most widespread method in use and seems likely to remain so, although the development of regressions of fine root biomass on some more accessible above ground parameter would simplify ecosystem studies.

Coring is always possible in organic soil or horizons but difficult or impossible in stony soils. This imposes constraints on the choice of study site and leads to bias in the information. Ingrowth methods (Persson, 1983) give interesting data but as yet we do not know exactly what is being measured. Ingrowth methods using undisturbed soil (Hamzah et al., 1983) are preferable in some respects, but the data are likely to be confounded by the length of time taken for excised root fragments to die (Ferrier and Alexander, 1984).

Core size

Fine roots and mycorrhizas are not uniformly dispersed in a sampling area. By the very nature of their morphogenesis they are likely to occur in aggregates. In addition there is the pattern, as yet not fully understood (but see Deans, 1979; Harvey et al., 1976; Reynolds, 1975) imposed by local variation in substrate, moisture, nutrient availability etc. This pattern exists in both space and time.

It is important to choose a core size which accommodates this pattern. Table 1 shows the effect of core size on estimates of mycorrhizal numbers in the forest floor of a 36 year old Picea sitchensis plantation. Variance changes with core size, good evidence that mycorrhizas are not randomly distributed. In this instance the "optimum" core size is 39 mm; there is no reason why that should be so on every site or indeed at all depths in the same profile. Reynolds (op. cit.) recognised a distribution pattern of Pseudotsuga menzesii fine roots conforming to cells around 55-75 cm in diameter. A smaller scale pattern is evident in Table 1.

There is no doubt that an appreciation of what determines local variation in mycorrhizal numbers and biomass, and the scale on which this variation occurs would allow more sensitive sampling procedures to be developed. In this respect some of the numerical methods designed for the analysis of pattern in terrestrial vegetation could usefully be applied to populations of mycorrhizas.

The number of samples

Spatial variation in mycorrhizal density has further implications for sampling - the number of samples required to get a good estimate of the mean is generally found to be large. For example in Table 1, the variance at optimum core size predicts that 44 samples from a 0.04 ha plot are required to estimate the mean with 95% confidence limits of ± 10%. In the event only 15 cores could be processed in the time available. Over 24 monthly samples these gave 95% confidence limits from ± 16-35% (mean = ± 25%). During this time the estimated sample size for 95% confidence limits ranged from 40 to 188. Biomass (< 1 mm diam.) estimates were less variable and gave 95% confidence limits from ± 12-27%.

These data refer to the forest floor only; as mycorrhizal density decreases with depth in the mineral soil the difficulties of getting good estimates increase. Neither can experience from one site be readily transferred to another. In a P. sitchensis stand of similar age and yield class, only 30 miles away from that giving the above data, but where spatial heterogeneity in the forest floor is more pronounced, a sampling intensity of 12 cores per month is giving 95%

Table 1. The effect of core size on the coefficient of variation for mycorrhizal density in the forest floor of a Sitka spruce plantation (Data from Fairley, 1983).

	Diameter of core (mm)					
	12	22	39	49	84	113
No. of cores taken in a 0.04 ha plot	50	50	20	12	10	10
Mean no. of mycorrhizas per cc	7.2	9.3	6.9	6.9	6.7	6.9
Coefficient of variation (%)	106	76	34	38	40	57
Estimated sample size to give 95% confidence limits = ± 10% $\bar{x}$	431	221	44	55	61	125

confidence limits from ± 34-70% of the mean.

This degree of accuracy may be acceptable in an experimental situation where treatment effects are large and consistent. Where the aim is to quantify the mycorrhizal role in carbon and nutrient flow through ecosystems better estimates are required. This is most likely to be achieved in the short term through careful selection of site. The long term aim must be to identify the factors causing spatial variability and stratify the sample accordingly.

Sampling frequency

In the temperate zone most studies have demonstrated a seasonal pattern of fine root and mycorrhizal biomass. Growth occurs in spring and autumn. Differences in seasonal biomass patterns result from differing amounts of production at these times and differing amounts of mortality in summer and/or winter. These major seasonal changes can be detected by monthly sampling, preferably over at least 2 consecutive years. More frequent sampling to detect lesser fluctuations requires increased sampling intensity. This effort would be best made at those times of year when mycorrhizal growth and/or mortality is greatest and accompanied, if possible, by environmental monitoring to quantify the nature and extent of environmental control over mycorrhizal production and death.

Estimating production and mortality

The magnitude of the seasonal fluctuations recorded in fine root and mycorrhizal biomass implies considerable production and mortality. These fluxes have been calculated from sequential biomass estimates by a number of different methods. Fairley and Alexander (1984) have recently compared these methods and concluded that calculations based solely on live root biomass underestimate production. Better estimates are obtained from measurement of all fine root material (live + dead) but optimal estimates are derived from separate measurements of live and dead biomass (balancing transfers: Santantonio, 1979; McClaugherty et al., 1982), which in addition permit estimation of mortality and disappearance. In all these methods, cumulative estimates result from the sum of differences observed between a series of observations. Clearly, it is possible for considerable error to accumulate in this process. It is important therefore that only significant differences between individual biomass estimates should be included in the cumulative estimates. In their own study Fairley and Alexander (op.cit.) found that when balancing transfers, inclusion of all changes gave an estimate of production 1.4 times larger than that using only statistically significant changes.

There are two important practical considerations when balancing transfers. The first is that a simultaneous, but independent, measure of dead fine root and mycorrhizal disappearance should be made using the litter bag method. This serves as a useful check on estimates obtained from fine root biomass. The second consideration is the problem of extracting dead mycorrhizal material, much of which is highly fragmented, and clearly differentiating between living and dead mycorrhizas. Current morphological classifications must be regarded as interim measures while we await definitive physiological and ultrastructural information on the process of ageing and death of mycorrhizal tissue.

The fungal component

It is clearly of interest to quantify the amount of fungal tissue associated with mycorrhizas under field conditions. Such information would permit better estimates of carbon flux and a clearer picture of nutrient uptake. It is possible, though laborious, with current techniques to quantify sporocarps, sclerotia and even the fungal sheath but the major problem remains the all-important extramatrical mycelium.

There are in fact two discrete parts to the problem. The first is to reliably differentiate between living and dead hyphae. The range of methods now available (see Domsch et al., 1979) goes some way towards this and, in such an active field, further innovation is to be expected. The second part of the problem, distinguishing between saprophytic and mycorrhizal mycelium on a scale large enough to allow quantitative estimates, seems likely to be around for some time.

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MODELING THE ESTABLISHMENT AND DEVELOPMENT OF VA MYCORRHIZAL FUNGI IN THEIR HOSTS

by

N. C. Schenck, Moderator

Introduction

The purpose of this discussion is to evaluate modeling systems, especially those applied to the development of mycorrhizal fungi in their hosts. It is hoped that this meeting can increase awareness of the use of modeling and will result in its increased application in mycorrhizal research.

Modeling has been used in a number of disciplines for several years. In plant pathology, modeling has been employed to assess the development of disease in plant populations. Van der Plank (1963) was instrumental in introducing the use of models in this field and they have become a vital part of epidemiological studies. Models have proven a valuable tool in predicting disease extensiveness and assessing the relative importance of factors impinging on plant epidemics. Baker et al. (1967) pioneered modeling with soil-borne plant pathogenic fungi.

In modeling, or systems analysis, the premise is made that any state of a mycorrhizal plant can be expressed quantitatively at any one time and that changes which occur can be described in mathematical terms. This, of course, is idealistic and only time and considerable research would be able to prove if this is possible.

Several advantages are apparent to using modeling in mycorrhizal research. A single value, obtained from the model, which includes the parameters total root length, mycorrhizal root length, and the number of entry points by the endophyte can be used to make comparisons between different treatments, experiments, and even researchers. An accurate means of estimating the rate of growth of the fungus in the root can be determined. Factors affecting fungus growth in the root can be evaluated that could not be measured using the percentage of root colonization values alone. Factors affecting the initial development of the fungus in the soil can be separated from those factors affecting the growth in the root. In addition, models can use information from seedling mycorrhizal plants to predict results in mature plants.

Modeling with mycorrhizae is still in its infancy with some of the first efforts reported less than 10 years ago (Tinker, 1975). Three recent papers by Buwalda et al. (1982), Sanders and Sheikh (1983), and Walker and Smith (1984) present the latest "state-of-the-art" with mycorrhizal modeling. These three papers present slightly different approaches to establishing a model to estimate the fungus growth in the root. All three model systems have much in common. We are fortunate to have individuals participating in this discussion that either authored or coauthored the three papers mentioned above.

Session Comments

In their presentations, each speaker covered the specific advantages of their model in estimating growth of mycorrhizal fungi in the root. However, lack of time prevented detailed discussion among the speakers in regard to the comparative merits of each modeling system. Questions from the audience were raised in regard to applying models to whole ecosystems. It was pointed out that ecosystem models would be far more complex than the simple models presented. All speakers indicated the problems involved with actually measuring the growth rate of the fungus in the root. It was suggested that the in vivo, nondestructive method of Ames et al. (1982), using florescent microscopy, may be a useful tool for this purpose. A question was raised about the relative growth rate of mycorrhizal and nonmycorrhizal roots. The growth rates of both were considered generally the same with most hosts.

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MODELLING MYCORRHIZAL DEVELOPMENT

By

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Introduction

A great deal of information is available on the rate and extent of mycorrhizal development in root systems, but this information is highly empirical and unrelated to mechanism. Within the last few years different groups have attempted to model mycorrhizal development. This has three aims: firstly to formulate and test hypotheses about how infection spread occurs; secondly, to develop methods for stating and handling this type of data more elegantly and more effectively, and thirdly, to allow prediction of how infection will develop in different conditions. Modelling is ideal for dealing with dynamic systems in which two organisms interact, as they do in mycorrhizal roots when both roots and fungi are growing. I stress that modelling is about ideas, not mathematics, which is simply the tool used in the modelling. It involves setting up hypotheses about the way in which the modelled process occurs, about the rate-limiting steps, and how different parts of the whole process interact. These hypotheses have to be stated in quantitative and exact form, as equations which can be manipulated, but the mathematics should always be kept as simple as possible. This paper reviews the models produced by the Rothamsted group with particular stress on the successive underlying hypotheses.

First model

The first published model was that of Tinker (1975). The underlying hypotheses in this was that the rate-controlling step was the frequency with which hyphae encountered uninfected parts of the root system, which was assumed to be proportional to the frequency of formation of new infections. It was assumed in these models that inoculum was 'placed', under the plant so that only infection spread, and not infection from propagules, was considered. Such encounters were taken to be proportional to the mean density of external hyphae in any soil volume (eg. a pot), which were assumed to be proportional to the length of infected root, L_i (Sanders et al. 1977). Secondly, 'successful' encounters will be proportional to the length of uninfected root, as enounters with infected roots will be ineffective, or at least will not be observed by normal techniques. This length is clearly $(L_t - L_i)$ where L_t is total root length in the same volume. Hence spread rates

$$\frac{dL_i}{dt} = S \cdot L_i (L_t - L_i)$$

where S is a constant. However, it was noted that infection virtually never reaches 100% of length, and often appears to stabilize at a fraction of L_t considerably below one. Hence this fraction is introduced empirically in a term n:

$$\frac{dL_i}{dt} = (SL_i (nLt - L_i) \qquad (1)$$

An additional elaboration in this model was that the root lenght L_t was assumed to be growing logarithmically, so that

$$L_t = ae^{Rt},$$

where a is a constant and R is the relative growth rate. This permitted an analytical solution of the equation, though a very complex one. Such an elaboration is unnecessary, and introduces a needless constraint when computer simulation methods can be used.

This concept implies that increased density of roots would increase infection. It later became clear that this is not true. Further, simple observation shows that infection forms discrete lengths ('infection segments') which are clearly separated from each other. Infection is thus much more likely to develop in root immediately adjacent to such a segment than in the average uninfected root. The next model (Buwalda et al. 1982a) therefore assumed that infection develops by spread from the 'fronts' bounding the segments. On average the number of fronts was taken to be proportional to the length of infected root in the defined volume. However, the chance of this spread actually producing measurable new infection depends upon it being into uninfected root, so

$$\frac{dL_i}{dt} = S'L_i \frac{L_t - L_i}{L_t}$$

The term n is inserted again for the same reason as above, so

$$\frac{dL_i}{dt} = S'L_i \left(1 - \frac{L_i}{nL_t}\right) \qquad (2)$$

This equation was extensively tested on plants grown at different densities, and shown to give excellent fit, whereas equation (1) gave a poorer fit when tested on a logarithmic plot.

Equation (2) is formally identical with the logistic equation, except that the term corresponding to nL_t would normally be a constant, where here it is a time-dependent variable. Smith & Walker (1981) published a model based on a different argument, but which led to an equation which again had considerable formal similarity with (2). Sanders & Sheikh (1983) developed a more extensive and complex model, which included propagule density and host response to infection. Equation (2) has been used by Buwalda et al. (1982b) to analyse the effects of different levels of phosphorus, who showed that this effect is almost entirely on the value of n in the model. We thus consider it to be a good 'functional' model, in the sense that it is useful for fitting and interpreting experimental data, and may be used for short-term prediction. The main uncertainty in this model lies in the physical and biological significance of the term S'. In a formal sense

$$S' = \frac{1}{L_i} \frac{dL_i}{dt}$$

when infection is just beginning, so that S' represents the relative growth rate of the fungus then. This seems to be the best

interpretation in conditions when the initial inoculum is strong and is placed near the base of the plant. If there is any delay in initial infection due to low inoculum levels, then this will clearly affect the value of S'. S' may best be looked at as a complex of all factors determining the speed of initial establishment and spread of infection.

There was thus still uncertainty about the underlying ideas of equation (2), so these were tested again by growing leek plants in 40 cm - deep pots, so that the root system could extend freely during the experimental period (Buwalda et al. 1984). Leeks were grown with both inoculum placed under the seedling, and dispersed throughout the pots. The assumption given above would predict that infection should spread at greater rates with dispersed inoculum, because this would cause more infectiion segments, and hence more fronts. This latter point was confirmed, but despite this the rate of spread of infection in both treatments was surprisingly similar. When the infection in single roots was observed separately, it was found to spread at a rate which was only slightly dependent on the number of fronts. It was concluded from this that there is some controlling factor within the roots which defines the total rate of spread of the endotype. This factor could be the supply of carbohydrates, but there is no direct evidence for this.

On the basis of this result, another model was constructed, which assumed that following a set delay time, infection spread at a uniform rate in each root. The lag infection arises from infection spreading steadily in all roots, and the 'plateau' where factorial infection approaches n, is given by the limiting ratio between speed of root growth

$$\frac{dL}{dt}$$

and rate of spread of infected root.

$$\frac{dL_i}{dt}$$

On this basis the spread of infection was successfully modelled, and also the way in which infection segment lengths were distributed in presence of dispersed inoculum (Buwalda et al. 1984). If this model is verified by further work, it leaves us with the very interesting question of the nature of the control which determines the total rate of spread within any one root. The most important result is that it gives a real meaning to n. This model could only have been developed for a simple unbranched root system such as leek; it leaves unclear whether similar rules govern spread within a branching root system. However, this third model is not 'functional' in the same sense that equation (2) is, because the measurements needed are far too elaborate to use routinely. It has great value because it allows us to develop our ideas of how processes work, whereas the practical models for handling and using data will be of the type of equation (2). In this paper I have deliberately not introduced the question of how these various equations or models are solved or handled. This is described in the original papers, and is important and often complex. Nevertheless, the most interesting aspect of modelling is always the underlying ideas, which I have discussed here.

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THE USE AND APPLICATION OF MODELING SYSTEMS TO THE STUDY OF VA MYCORRHIZAL FUNGI

By

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Keywords--Modeling, Inoculum potential

As a field oriented researcher, I view modelling as another scientific tool with which we can seek practical answers to practical questions. Using models described by Buwalda et al., 1982 or Smith and Walker, 1981, it is possible to characterize infection curves for a particular VAM fungus on a particular host under specific conditions. Using the formula:

$$\frac{dLi}{dt} = SL : \frac{(1-Li)}{nLt}$$

developed by Buwalda et al., 1982, where Li = length of infected roots, dt = change in time, S = maximum growth rate of the fungus, n = maximum % infection of a given time, and Lt = length of root at time t, it is possible to assign values for S and n under specific conditions and then solve for an infection level at anytime. Current evidence indicates that vesicular-arbuscular mycorrhizal (VAM) growth responses are a result of P uptake. P uptake appears to be directly correlated to the amount of external hyphae. External hyphae are partially a characterisitic of the VAM isolate but are also strongly a function of infection level. Hence external hyphae and P uptake could be predicted once reliable infection predictions can be made.

My particular interest in VAM infection models stems from the knowledge that infection curves are strongly influenced by inoculum potential. While the maximum growth rate of the fungus (S) and the maximum % infection (n) do not appear to be influenced by inoculum potentials, the length of the lag phase before logarithmic increase in infection is due primarily to the inoculum potential of the VAM inoculum. This lag phase is critical since it can shift the entire infection curve one way or the other by as much as two months in some crops. By shifting the infection curve we have a powerful tool with which we can regulate the amount of infection and hence the timing for P uptake. Current models for VAM infection do not adequately account for the inoculum potential. A formula such as:

$$\frac{dLi}{dt} = \frac{[Ip]\,[Lt]}{K + Li(T)} + \frac{SLi(1-Li)}{nLi}$$

where Ip = inoculum potential at time 0 in infective units/gm soil, K = constant, and T = time after inoculation, would provide a starting point and an initial infection rate for the standard infection model. The effect of inoculum potential would diminish with time and root infection. Most importantly after inoculum potential become integrated into the model we can begin to get some idea about the relationship between inoculum potentials and infection at a given time. Armed with this knowledge a field oriented researcher may begin to recommend inoculum amounts for field use. These recommendations must still be tempered with judgement but at least they will have a theoretical basis, which can be modified by experience. These recommendations may allow us to regulate the time of maximum P uptake.

Applying models such as these to citrus under field conditions presents many intriguing problems, but it is a practical as well as intellectual pursuit. This effort has already led to information on the proper time to inoculate citrus and on the amount of inoculum to use.

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THE USEFULNESS OF CURRENT MODELS OF V.A. MYCORRHIZAL INFECTION

By

N. A. Walker and S. E. Smith

It is generally recognised that comparison of the way symbioses between different pairs of organisms function under different conditions requires a quantitative consideration of the way infection develops.

Conventionally, progress of infection has been measured in terms of the proportion of the root length infected (% infection). This is a convenient parameter to measure on samples of root and is considered by many plant nutritionalists to be of paramount importance in consideration of nutrient uptake. However, use of % infection obscures the fact that environmental factors may act separately on (i) plant growth, (ii) fungal infection and (iii) fungal growth. The rate of infection is very important in determining mycorrhizal effects on plant growth, so that quantitative comparisons of spread of infection independent of the growth of the host are required.

The mathematical models of V.A. mycorrhizal infection recently developed have all had as one of their aims the calculation of parameters which are not immediately obtainable from measurements of % infection (Buwalda *et al.* 1982 a & b; Buwalda *et al.* 1984; Sanders and Sheikh 1983; Smith and Walker 1981; Walker and Smith 1984). The models differ in what these parameters are and underlying these differences are differences in conception of the way infection develops and the degree to which it is possible or desirable to separate spread of infection into its component processes. They all have strengths and weaknesses which it is worthwhile considering.

Our model (Smith and Walker 1981) was explicitly designed to calculate two parameters: the frequency of infection of roots (A) and the rate of growth of infection units in the root cortex (B). The model uses two differential equations, shown here in their most straightforward form:

$$dU/dt = A(L-L^*) \qquad (1)$$

$$dL^*/dt = BU(1-L^*/L) \qquad (2)$$

Where U is the number of discrete entry points, L is the length of root per plant and L^* the infected length of root per plant. Use of the model requires measurements of these variables. We considered that entry points could occur on any uninfected region of root, and the term $(L-L^*)$ was included to allow for this. Distribution of inoculum throughout the rooting medium means that there is no constraint on the position of infection units caused by localised placement. The calculation of both A and B is limited to very young plants because it is necessary to determine the number of entry-points (U). This becomes impossible as the root systems increase in length and branch, and as infection units come to overlap more extensively. Overlapping at short times is allowed for in the term $(1-L^*/L)$ but our approach can still only be used where individual entry-points are distinguishable. The growth in length of roots is taken into account in the calculations and actual measurements of root length are used for this, fitted by suitable spline or polynomial curves. This is another constraint to the use of our model and others, as measurement of the whole root system is required. Only the preliminary model of Tinker (1975) assumed a particular (exponential) model for growth of roots, although Buwalda *et al.* (1984) have also used an exponential as well as a logistic curve to fit their data. Values of A and B obtained using our model are average values for that part of the root system for which data is lumped. No prediction of the spatial distribution of formation of new entry-points on particular regions of the root is made: this is left for experimental study. We have found that there is a spatial variation in A, which is lower (by a factor near two) on main roots than on lateral roots of *Trifolium subterraneum*. The determination of the spatial distribution of new entry-points along roots is important for a refining of the model, a process which simultaneously refines our view of the infection process itself. We have made no attempt so far to model A and B as functions of time but, within the constraints of short experiments and the difficulty of obtaining data, we can observe how the average values of these parameters change with time. We have as yet made no systematic investigation of these time courses. At short times, A appears to be linearly related to the density of uniformly distributed propagules in soil. At longer times and high densities this linear relationship no longer holds and it seems likely that formation of secondary entry-points complicates the picture. B is not dependent upon propagule density and its average value appears to decline with time, strongly in main roots. The apparent mean length of an infection unit in a population of infection units appears to reach a plateau, at least for main roots which stop growing. This was predicted also for growing roots (Sanders and Sheikh 1983) assuming a steady rate of growth of each infection unit and an exponential rate of formation of new infection units. We have not found an exponential relationship in our experiments, nor did Sanders and Sheikh use this exponential model in their consideration of spread of infection in roots. In any case, there would not be general agreement about a steady rate of growth of each infection unit, for Tinker (1975) and Buwalda *et al.* (1982 a & b) model infection as though each infection unit grows instantaneously to a maximum length. Both the assumptions of steady growth and 'instantaneous' growth must be oversimplifications and attempts to model the age structure of populations of infection units (or discrete lengths of infected root) should help to define more precisely the growth of individual units (Sanders and Sheikh 1983; Walker and Smith 1984; Buwalda *et al.* 1984).

Models in which entry-points or individual infection units are considered can only be applicable at short times. A more generally applicable model was developed by Buwalda *et al.* 1982 a & b. They used data for root length and infected root length to calculate parameters, which were considered to represent "definite characteristics of the host/endophyte/environment system". The maximum fraction of the root infected n was included as a correction term to account for the fact that the fraction of the root length infected rarely reaches 1.0, while S was considered

to be an important parameter relating to the spread of fungus and useful for comparison of fungal behaviour under different conditions. Buwalda *et al.* considered that the correction factor *n* was important as it was related to the control they believed to be exercised by the host, following an earlier phase during which spread of the fungus was essentially independent of the amount of root tissue available for colonisation. This model had advantages as it could be applied at long times, but disadvantages in that (i) the total root length was required, (ii) the parameters *S* and *n* were poorly defined in biological terms.

More recently Buwalda *et al.* (1984) have recognised these problems, particularly that of the definition of *S*. Young leek plants were shown to be very suitable material for study of early infection because it was possible to determine the rate of growth of individual adventitious roots at the same time as measurement of lengthwise spread of infection in each root. The results indicate that both the rate of growth in length of individual roots and the rate of spread of infection in each root can be reasonably closely fitted by linear functions. This finding led Buwalda *et al.* to the conclusion that spread of infection is determined by rate of root growth, even at very early times, and confirmed their belief that the parameter *n* is of central importance in the development of infection. Placement of inoculum had little effect on the rate of spread of infection in single roots, although the number of fronts of infection was greater when the inoculum was dispersed throughout the soil profile. Thus, the greater the number of fronts, the slower they apparently all grew. It would be interesting to investigate the effect of varying the amount of inoculum on infection in this system, as it is possible that the apparent 'host-control' on infection and the indication that both fungal growth and host growth are constant, may be a result produced under conditions of 'saturating' inoculum.

Elegant though the leek system is, it is subject to the same criticisms of use of young plants as our own work and indeed does not tackle the prob- of what happens as the lateral roots begin to be produced. Clearly differences in rooting pattern may affect the way in which placement of inoculum interacts with root growth and may explain differences between results obtained with clover and leek plants which produce lateral roots from about 8 days and 42 days respectively.

In summary: the recent attempts at modelling infection have been partially successful using young plants - but all are inapplicable to older plants and field material. It is clear that density of propagules in soil does affect the frequency of formation of entry-points at very short times, but that much more work is required to unravel what happens at longer times. We need to know considerably more about the susceptibility of different regions of the root systems both to infection from the soil and to growth of infection units or fronts within the cortex. We have already demonstrated that main and lateral roots of clover appear to be differently infectable on average. It seems likely that consideration of rates of linear extension of roots of different types and on different species may be useful, as it has been with the work on leek. The growth of individual infection units or sections of infected root remains a grey area which requires further consideration. It seems unlikely that infection obeys the "rules of infection" laid down recently by Buwalda *et al.* (1984). It is certainly not true that overlap does not occur and it is hard to believe that when fronts touch they cease to grow and the exposed fronts then double in speed. These rules may seem far-fetched, but they will act as a challenge - so that mycorrhizasts are provoked into making careful observations and experiments on these important aspects of infection. From the point of view of the physiology of infection the activity of infections (as well as their extent as revealed by staining) must also be considered in future work.

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PHYSIOLOGY OF MYCORRHIZAE

THE CONCEPT OF EFFECTIVENESS IN SYMBIOTIC RELATIONSHIPS

By

S. E. Smith

The concept of effectiveness in a symbiotic association is complex and implies that there is considerable variation among the organisms capable of forming a particular kind of association. This range of behaviour is well documented for example in the legume/*Rhizobium* symbiosis, where the variability is emphasised by the fact that not all legumes are susceptible to infection by all rhizobia and it is possible to group organisms into particular cross-inoculation groups. It is clearly recognised that the interactions between host and rhizobia during nodule formation are extremely complex; the ability to form effective, nitrogen-fixing nodules being controlled by genetic and physiological factors operating at many points during development (see Sprent, 1979; Vincent, 1980). For mycorrhizal symbioses the concept of effectiveness is much less clearly defined and the word "effective" certainly suggests different ideas to different people. This reflects greater ignorance of mycorrhizal symbioses compared with nitrogen-fixing systems and also the fact that mycorrhizas have indeterminate growth (compared with nodules) and less clearly defined physiological effects, as well as wider host ranges. Mycorrhizal effectiveness cannot be defined in terms of weight of nodules or amount of nitrogen fixed. Nevertheless, if the concept of effectiveness in mycorrhizal systems is to be useful then it must be defined in terms of measurable parameters so that the behaviour of different symbionts can be assessed accurately. Appropriate parameters must be chosen, depending on which aspect of symbiotic effectiveness is considered and what the experiments are designed to investigate.

The speakers in this symposium are mainly interested in vesicular-arbuscular mycorrhizas, but I hope that what they say will be applicable to other types of mycorrhiza. It seems likely that the first advances in some aspects of the physiology and genetics of integration between mycorrhizal symbionts will be easier in examples where we know something of the sexual reproduction of the fungi and also in cases where the fungi can be cultured: that is in ecto-mycorrhizas, ericoid mycorrhizas and orchid mycorrhizas. Of course, information obtained from organisms in pure culture will have to be treated with the usual caution when it comes to consideration of symbiotic development and we must not necessarily expect the factors controlling this development to be the same in different types of mycorrhiza.

Up to the present the term "effectiveness" in mycorrhizal association has been used in different ways by different people. For many the statement that one mycorrhizal fungus is more effective than another immediately implies that it increases the growth of a host plant to a greater extent. It is clearly recognised that this statement must be qualified in terms of the species of host plant, the soil fertility and other environmental conditions. It is also being increasingly realised that the source and quantity of the fungal inoculum must be controlled as comparisons between different fungi can be confused if external factors (rather than inherent behaviour of the fungi themselves) affect the rate of development of infection (e.g. Luedders *et al.* 1979). More and more investigators include consideration of the effects of different propagule densities in soil and "inoculum potential" of different fungi (e.g. Daniels *et al.* 1981; Carling *et al.* 1979; Walker & Smith 1984). Unfortunately some past work comparing growth of plants inoculated with different fungi neither considered the problems of controlling amounts of inoculum nor monitored the rate of plant growth or development of infection during the course of the experiments. These problems aside, measurement of effectiveness of a symbiosis in terms of plant growth can be useful in some contexts. The rate at which plants grow, the time at which they attain maturity and the seed yield are all relatively easy to measure. These parameters can be very relevant for agronomists, crop physiologists or higher plant ecologists who are interested in crop yields or establishment and spread of species in natural communities. However, measurement of yield alone is unlikely to improve our knowledge of the genetic and physiological mechanisms which underlay the growth responses. Even if measurements are restricted to the dry-matter production and mycorrhizal infection of a plant, a great deal more information can be gained if rate of growth is calculated from data for several harvests, rather than a single harvest, and extent of mycorrhizal infection and contribution of roots to the dry-matter production of the plant is also considered.

Measurements of host plant growth are not only used in assessing the effectiveness of different fungi, they have also been used to determine the differences in response of different species or varieties of host plant to infection by a single mycorrhizal fungus. Such differences in response have been referred to as differences in "mycotrophy" (e.g. Baylis 1972) or "mycorrhizal dependency" (e.g. Gerdemann 1975; Menge *et al.* 1978) or "mycorrhizal efficiency ratio" (Powell and Daniel 1978). Again these measurements must be qualified according to environmental conditions. Gerdemann certainly defined mycorrhizal dependency as "the degree to which a plant is dependent on the mycorrhizal condition to produce its maximum growth at a given level of soil fertility". Those interested in crop yield and establishment of plants may find these parameters useful. However, the variability among host plants in responsiveness to mycorrhizal infection (see Fig. 1) brings us to one of the problems of considering effectiveness of a symbiosis in terms of the growth of only one of the partners in the association. Choice of host plant will certainly affect the quantitative estimate of the effectiveness of particular fungal species. For example, comparison of the response of a plant with low mycorrhizal dependency (such as oats) to inoculation with several different fungi, might lead to the conclusion that all were ineffective because growth of the host was not increased. A similar

conclusion would be drawn if a highly-dependent host plant were grown on fertile soil. However, if a dependent host plant such as clover, was used then differences between the fungal species might become apparent. Fig.1 shows mycorrhizal dependency of several species calculated on the basis of shoot weight as well as of total plant weight. It is interesting that the alterations in the root:shoot ratio following infection can mean that increase in total plant growth is actually much less than might be expected if shoots alone are considered. This does not mean that the mycorrhizal fungi are having no effect on the plant. Mycorrhizal infection can become established and nutrient uptake increased under conditions which, because of the choice of plant species, soil conditions or use of whole plant dry matter as the parameter chosen to measure "effectiveness", the conclusion would be that the symbiosis was ineffective. However, if other parameters such as phosphate uptake or transfer to the host were used as criterion of effectiveness a different conclusion about the fungi might be reached. P uptake into mycorrhizal plants can be greater than into non-mycorrhizal plants even under conditions where P supply does not limit the growth of the plants, as shown by the higher concentration of P often found in tissues of the mycorrhizal plants (e.g. Stribley *et al.* 1980; Pairunan *et al.* 1980) and by calculation of P inflow from soils containing different amounts of P (Smith 1982).

To other mycorrhizasts less interested in the growth of host plants the term "effective" might imply that the fungus in question was successful at surviving in soil, in colonising roots from soil, in competing with other organisms including other mycorrhizal fungi for space within the root, or perhaps successful in producing large amounts of external hyphae, fruit-bodies or spores. It would be as valid to consider the effectiveness of mycorrhizal symbiosis in terms of these fungal parameters as by measurements of growth of host plants. Indeed for those interested in truffle production the effectiveness of a symbiosis might most usefully be measured in terms of growth of the fungus and production of fruit bodies. This approach has rarely been taken, partly because fewer people are interested in the agricultural productivity of the fungi, but also because it is hard to quantify the amount of fungal tissue in a particular mycorrhizal association (see Harley & Smith, 1983 for references).

In any case, measurement of effectiveness of an association in terms of the productivity of one or other of the organisms overlooks the mutualistic nature of mycorrhizal symbioses and provides little information on the mechanisms underlying differences in productivity between different pairs of organisms. It must not be forgotten that nutrient transfer occurs bi-directionally between the two organisms so that their structure, physiology and biochemistry must

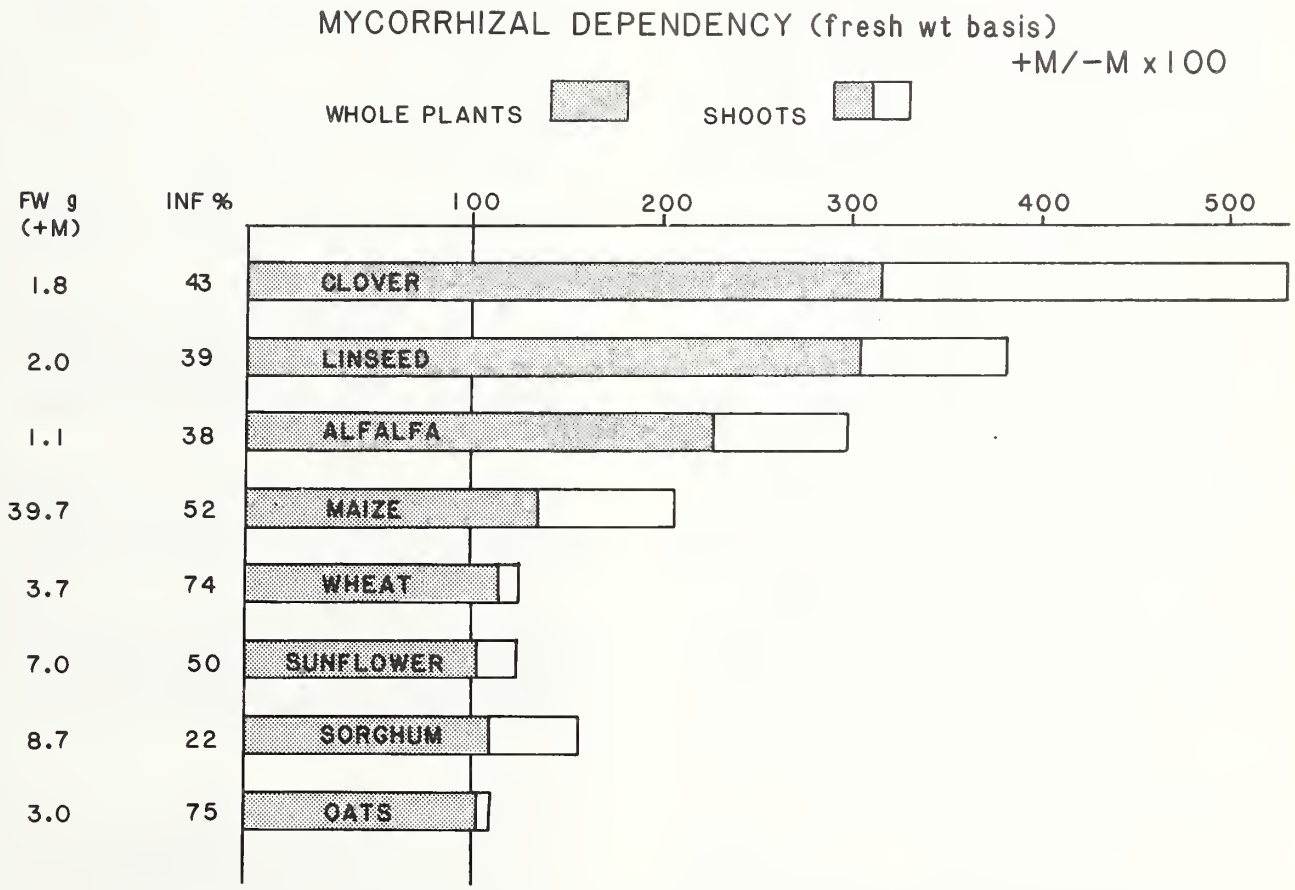

Fig.1 Fresh weight of mycorrhizal plants, percentage of root length infected and mycorrhizal dependency of a range of crops grown in low P soil. Mycorrhizal dependency calculated on the basis of roots + shoots or shoots only.

be integrated. The effectiveness of this integration and the efficiency of bidirectional transfer of nutrients must, in the last analysis, determine the productivity of the organisms and their ability to grow in particular environments.

The topics to be considered in this symposium are concerned with particular aspects of the physiology of mycorrhizal plants. I hope that they will take us towards quantitative determination of which factors may be rate limiting in the bidirectional transfer of nutrients. If the rate-limiting steps can be identified, they may then indicate the physiological parameters which must be measured in order to determine differences in efficiency between different pairs of symbionts.

The kinds of quantitative information required will include both details of biochemistry and physiology of the associations and also details of anatomical and morphological development of infection. The structural information is required for at least two reasons. Firstly, it must be available so that physiological measurements can be put on a sound and relevant quantitative basis, and, secondly, because anatomical and developmental details can provide information on stages in recognition and mutual structural alteration between symbionts. The measurement of nutrient uptake provides one example of how changes in the basis on which results are expressed have improved, and could still further improve, our understanding of mycorrhizal effects. Early work showed that mycorrhizal plants frequently contained higher concentrations of nutrients (particularly phosphate) than non-mycorrhizal controls. This finding was taken at its face value and provided the impetus for further work on the importance of mycorrhizal infection to uptake of phosphate by plants. For some time it was considered that mycorrhizal fungi could only have affected uptake if tissue concentrations were elevated. This has now been shown to be an over-simplification. Certainly, elevated nutrient concentrations in the tissues do indicate increased uptake. However, alterations in uptake can be more directly demonstrated by calculations of the rates of uptake on the basis of root weight or root length, as these measurements are independent of changes in root:shoot ratio which so often occur following mycorrhizal infection. With the emphasis removed from tissue concentration as an indicator of uptake, the realisation came that these elevated levels could only occur if some factor other than the nutrient in question was limiting growth (Smith 1980; Stribley *et al.* 1980). Complex interactions in host/fungus physiology have thus been highlighted. Attempts have been made to calculate the proportion of uptake into vesicular-arbuscular mycorrhizal plants due to the fungus (e.g. Sanders *et al.* 1978) so that different fungi could be compared. However, the calculations are complicated by indirect effects of improved phosphate nutrition on the metabolism of the host. Nevertheless, extension of uptake studies together with such measurements as length of living hyphae outside the root, area of contact between symbionts and attempts to estimate the proportion of the infection in the root which is active are likely to provide much improved bases for comparison of nutrient uptake mediated by different fungi. Techniques are certainly available for comparison of the rates of translocation of nutrients by different fungi.

Study of a single nutrient in isolation can only be of limited use in investigation of the complex interaction between two symbionts. It has been suggested that the supply of photosynthate may limit the growth of mycorrhizal plants which have elevated phosphate concentrations in the tissues (Stribley *et al.* 1980). If true, this indicates that while growth of the host is restricted, the activities of the fungus continue, at least in terms of the energy-requiring processes of phosphate uptake, translocation and transfer to the host. Calculation of phosphate absorbed by the fungi per unit of carbohydrate utilised might be most useful, not only in selecting fungi capable of efficient phosphate acquisition, but also minimal carbohydrate use.

Interactions between mycorrhizal infection, phosphate nutrition and nitrogen nutrition are now gaining more attention. Ecto and ericoid mycorrhizas have long been recognised as having importance in nitrogen nutrition, particularly in uptake and assimilation of ammonium (see Bowen & Smith 1981 for references) and work is now in progress on the fungal enzyme systems that may be involved (e.g. Martin *et al.* 1983, St.John *et al.* in preparation). Recently Ames *et al.* (1983) demonstrated that hyphae of V.A. mycorrhizal fungi may also be directly involved in the ammonium nutrition of host plants and the presence of glutamine synthetase has been detected in mycelium, vesicles and arbuscules of *Glomus mosseae*, separated from onion roots (Smith *et al.* in preparation). The activity of enzymes of ammonium (glutamine synthetase), nitrate (nitrate reductase) and nitrogen (nitrogenase) assimilation are certainly affected by phosphate supply (e.g. Smith *et al.* in preparation; Carling *et al.* 1978; Oliver *et al.* 1983; Asimi *et al.* 1980) so that indirect as well as direct effects of mycorrhizal infection are very important in the integrated responses of plants to infection by mycorrhizal fungi. We know very little indeed about how fungal metabolism may be affected following establishment of symbiosis.

Studies of the detailed anatomy of the symbionts and changes that take place as infection is initiated, matures and later senesces are also required in order to help identify the stages at which important events of recognition and physiological integration are likely to occur. These events may well be under genetic control in both organisms (as similar events are in *Rhizobium*/legume symbioses) and it is these genetic differences which must underlay any observed differences in "effectiveness".

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MYCORRHIZAL EFFECTIVENESS IN PHOSPHATE NUTRITION : HOW, WHEN AND WHERE ?

By

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Keywords-- plant dependency, fungal efficiency, soil P, phosphatases, P accumulation, biochemical markers.

The effectiveness of mycorrhizae in the phosphate nutrition of plants depends on a series of complex interactions between the plant's capacity to satisfy its P requirements (mycorrhizal dependency), the ability of the fungus to infect and provide P to the plant (fungal efficiency), and the relative amounts of different forms of P in the rooting medium. Any factors affecting the processes involved in the interactions between these different components will determine the magnitude of the mycorrhizal effect on P assimilation.

A prerequisite to understanding mycorrhizal effectiveness in phosphate nutrition, therefore, is a sound knowledge of how the mycorrhizal system functions. Advances that have been made in this field differ considerably from one type of mycorrhiza to another. Practically nothing is known for ericoid and orchid mycorrhizae as compared to VA mycorrhizae or ectomycorrhizae whilst much of the research on P uptake by the latter has, contrary to that concerning VA mycorrhizae, used excised mycorrhizae where the external soil phase mycelium is eliminated.

Variations in mycorrhizal infection development and dependency are greater in VA mycorrhizal plants than in plants forming other types of mycorrhizae and they have been illustrated at the level of species, variety/cultivar, clone (see Gianinazzi-Pearson, 1984 for references) or ploidy (Gianinazzi, 1982). However, it is not always clear when there is a lack of response to mycorrhiza whether this is due to an association being really ineffective in phosphate uptake or whether it is because other factors are limiting responses to improved phosphate supply to the plant (Trouvelot *et al*, 1982). Root morphology and susceptibility to infection, growth rate, phosphate requirement, root P absorbing power, uptake mechanisms and seed reserves are all factors that may be involved in determining mycorrhizal dependency ; these will not necessarily be the same for annuals, perennials, herbaceous or woody plants. Only the use of genetically well-defined plant material will enable real advances to be made in this area.

Although there is now no doubt that mycorrhizae are more efficient than non-mycorrhizal roots in the absorption of soluble soil phosphate, confusion still exists about their role in P uptake from insoluble P sources. ^{32}P-labelling experiments have repeatedly shown that in unamended soils mycorrhizae absorb from the labile pool of the native soil P, as non-mycorrhizal roots do (see Harley and Smith, 1983 for references). There is no convincing experimental evidence that VA mycorrhizal infection improves the ability of roots to degrade insoluble inorganic phosphate fertilizer (Pairunan *et al.*, 1980 ; Gianinazzi-Pearson *et al.*, 1981). In fact, VA mycorrhizae do not significantly increase P inflow rates into roots with increasing insoluble fertilizer doses (Table 1) nor improve ^{32}P absorption from a ^{32}P-labelled insoluble phosphate source (Table 2). Similar studies will clarify this point for other types of mycorrhizae.

Table 1. Phosphate inflow(mol.cm $^{-1}$ s $^{-1}$ X10^{15}) to mycorrhizal (M) and non-mycorrhizal (NM) roots of *T. subterraneum* in a P-deficient soil/sand mixture amended with $Ca_3(PO_4)_2$ (S.E. Smith, unpublished results).

Time interval (days)		Additions (mequiv. phosphate kg^{-1}) as $Ca_3(PO_4)_2$		
		0	0.1	0.6
0-22	NM	6.1	2.0	3.1
	M	7.7	4.7	6.3
22-29	NM	-ve	3.3	1.7
	M	6.1	3.9	4.7
29-36	NM	2.4	1.2	2.3
	M	3.9	5.0	5.1

Table 2. Ratio of ^{32}P to ^{31}P in mycorrhizal (M) and non-mycorrhizal (NM) soybeans growing in a low P soil (24ppm Olsen P) amended with $Ca_3(^{32}PO_4)_2$ (C. Azcon, V. Gianinazzi-Pearson, J.C.-Fardeau and S. Gianinazzi, unpublished results).

Treatment	Additions (mequiv. phosphate kg^{-1}) as $Ca_3(PO_4)_2$	
	6	30
NM	0.23	1.06
M_1 (*G.mosseae*)	0.19	1.00
M_2 (*Glomus* E_3)	0.17	0.82

The formation of mycorrhizae may, on the contrary, make roots more active in the breakdown of certain soluble phosphates and insoluble organic phosphates like phytates (Gianinazzi-Pearson *et al.*, 1981 ; Azcon *et al.*, 1982). The few comparative studies on surface phosphatase activities of mycorrhizal and non-mycorrhizal roots indicate that these can be modified quantitatively by mycorrhiza formation (Gianinazzi-Pearson and Gianinazzi, 1981 ; Doumas, 1984 ; Table 3) ; more research is necessary to confirm the possible role of different types of mycorrhizae in the

Table 3. Effect of mycorrhiza formation by Pisolithus tinctorius on surface acid phosphatase activities (n mol. P released min^{-1} g^{-1} f.wt) of Pinus pinaster roots (Doumas, 1984).

Substrate	Nonmycorrhizal	Mycorrhizal
p-nitrophenyl phosphate	28.3	35.8
pyrophosphate	10.4	41.2
tripolyphosphate	6.8	17.0
phytate	6.1	13.3

Table 4 : Acid phosphatase activities (μ mol. phosphate released min^{-1} g^{-1} d.wt) of ten ectomycorrhizal fungi (D. Mousain, C. Polard and N. Bousquet, unpublished results)

Fungal isolate	pNPPase activity	Phytase activity
P. tinctorius F_{11}	120	0.36
" F_9	195	2.60
H. cylindrosporum D. 3.25/6	183	1.88
S. luteus J 12.21/5	117	0.27
" J 12.21/9	255	0.18
S. granulatus J7/10	519	0.10
" J7/5	643	3.95
S. bellinii J 2.5/3	469	0.06
S. bovinus J 2.15/4	286	0.15
" (Algeria)	241	0.31

mobilization of P from different forms of phosphate. In pure culture, ericoid and ectomycorrhizal fungi do possess enzymes that could contribute to P mobilisation but the ecological significance of these in situ needs to be determined.

Inter-and intraspecific differences exist in the ability of mycorrhizal fungi to improve the phosphate nutrition of host-plants. The fungal efficiency in phosphate uptake must depend not only on the fungus being able to colonize host tissues and extensively develop in the soil, but also on the capacity of the external mycelium to absorb P from the soil and transport it to the plant roots. In fact, infection intensity at plant harvest which is used as a measure of fungal development is not always proportional to the mycorrhizal effects ; it would be useful to follow the rate of root colonization as this may be a more important variable contributing to fungal efficiency. The little data available on the external mycelium in VA mycorrhizae shows that this increases during the active phase of internal mycelium development and that it is related to P uptake by the mycorrhizal plant (Sanders et al., 1977 ; Bethlenfalvay et al., 1982). More extensive studies are necessary on the internal and external development of different VA fungi in order to assess the relative importance of these two phases in phosphate nutrition during plant ontogeny. Ectomycorrhizal fungi show different degrees of development of external mycelium out from the sheath but the implications of this in their efficiency in phosphate nutrition have not been studied. The activity of the external mycelium in P absorption and translocation to mycorrhizal roots has been demonstrated in vitro and in vivo (see Harley and Smith, 1983 for references ; Hale and Sanders, 1982) and the relatively few studies on specific uptake rates or hyphal inflow of mycorrhizas formed by different fungi suggest that these may have different uptake efficiencies or transport capacities (Sanders et al., 1977 ; Langlois and Fortin, 1978) ; this could be easily confirmed by comparative in vitro measurements of P absorption and translocation by the different mycorrhizal fungi.

Knowledge of the processes involved in the hyphal pathway of P transfer from soil to mycorrhizal roots is essential as each of them can be a rate-limiting step in the overall absorption of P by mycorrhizal plants. Nothing is known of the absorbing properties of the external mycelium of mycorrhizae, for instance, where hyphae absorb or whether they have high affinity absorption sites for P. In ericoid mycorrhizae and ectomycorrhizae, however, external hyphae may have surface phosphatases as in pure culture mycelium (Pearson and Read, 1975 ; Calleja et al., 1980). The activity of phosphatases varies between ectomycorrhizal fungi (Calleja and d'Auzac, 1983 ; Table 4) and this may be a contributing factor to their efficiency in supplying the host plant with phosphate. Hyphae of mycorrhizal fungi can accumulate P by sequestering it into their vacuoles where it is converted into the condensed form of polyphosphate. Fungal sheaths of ectomycorrhizae can accumulate large quantities of P in this form when phosphate is available in the soil and mobilize this P for transfer to the plant when P supplies in the soil become deficient. Much higher concentrations of total P (C. Plenchette, 1982 ; Smith, 1982), orthophosphate and acid-labile phosphate (ATP, sugar phosphates, polyphosphates) have been found in VA mycorrhizal roots as compared to roots of nonmycorrhizal plants (Table 5) and orthophosphate levels increase steadily in mycorrhizal roots whereas they decline in roots of "matched" nonmycorrhizal plants growing in soil to which soluble phosphate has been added (Table 6). It is not impossible therefore that VA mycorrhizae, similarly to ectomycorrhizae, can have a (perhaps shorter-term) storage role for phosphate but extensive studies are necessary to confirm this.

Translocation of P along hyphae of mycorrhizal fungi is metabolically dependent (Skinner and Bowen, 1974 ; Pearson and Tinker, 1975 ; Cooper and Tinker, 1978) but what triggers P loss from them in presence of the cells of the host root

Table 5. Amounts (μg Pi.g $^{-1}$ f.wt) of orthophosphate and acid-labile phosphate in nonmycorrhizal (NM) and mycorrhizal (M) onion roots.

	Orthophosphate		Acid-labile phosphate(100°C, 1N acid, 7 min)	
Experiment :	1	2	1	2
NM	18.0	18.5	1.3	5.1
M :				
G. mosseae	-	40.4	-	23.2
G. fasciculatus	76.6	54.8	16.1	18.5
Glomus E_3	97.8	82.4	21.6	38.4

Table 6. Amounts (μg Pi.g $^{-1}$ f.wt) of orthophosphate (OP) and acid-labile phosphate (ALP) in roots of mycorrhizal (M) and non-mycorrhizal onions growing in soil with (NMP) and without (NM) added soluble phosphate.

	Weeks					
	4		6		8	
	OP	ALP	OP	ALP	OP	ALP
NM	18.4	7.6	16.6	5.3	19.7	6.2
NMP	116.3	9.6	77.6	10.3	59.1	9.7
M :						
Glomus E_3	39.2	32.8	66.4	38.6	129.9	43.7
(% infection)		63		82		92

is not known although it has been suggested that this may be coupled to carbohydrate assimilation by the fungus. If so, the release of fungal P could somehow be under control of the host plant. Large surface contact between fungus and host cell is a feature of all mycorrhizae ; ectomycorrhizal fungi develop opposite the existing plasmalemma whilst in endomycorrhizae the fungi provoke an important extension of this membrane. The plant plasmalemma is not adversely affected by the proximity of a mycorrhizal fungus (Gianinazzi-Pearson et al., 1984) ; the only modification that has been observed is the specific localisation of host plasmalemma-bound ATPase around fine arbuscule branches in VA mycorrhizae (Marx et al., 1982). Phosphate released by the mycorrhizal fungus can be absorbed by the host cell ; there is no reason to invoke special mechanisms for this P uptake by the root cells in mycorrhizae.

There is some evidence that the fungal associate may also influence the absorbing properties of the root tissues themselves ; for example, VA-infected roots freed of external mycelium have lower Km values at low P concentrations (Cress et al., 1979) and nonmycorrhizal roots on mycorrhizal plants have been reported to have higher uptake capacities than those on non-mycorrhizal plants (Gray and Gerdemann, 1969 ; Bowen et al., 1975). Such modifications would provide additional advantage to mycorrhizal plants during periods of phosphate release in the soil.

Amongst the different environmental conditions that can affect the effectiveness of different plant/fungal combinations on phosphate uptake, soil phosphorus levels are of particular importance. Mycorrhizal effects decrease with increasing levels of available phosphate in the rooting medium and infection is reduced in the presence of high amounts of P. At extreme levels of available phosphate in the rooting medium, the growth of nonmycorrhizal plants can exceed that of VA mycorrhizal ones (Smith, 1982 ; Bethlenfalvay et al. 1983 ; Kiernan et al. 1983) and for a same concentration of P in the tissues, VA mycorrhizal plants can have lower yields than nonmycorrhizal plants (Stribley et al., 1980 ; Pairunan et al., 1980). These yield reductions in mycorrhizal plants could be due as much to the increased metabolic activity of the infected root cells as to the suggested carbohydrate utilisation by the fungi (Stribley et al., 1980 ; Buwalda and Goh, 1982). However, there are often widely differing values for levels of P versus fungal colonization of roots, mycorrhizal dependency or fungal efficiency because of the different plants, fungi, growth conditions and rooting media used. There is an urgent need to standardize conditions for testing fungus/plant combinations and to develop methods for predicting mycorrhizal effectiveness in different soils (Plenchette, 1982 ; Ojala et al., 1983). The effect of phosphate supplies on root colonization can vary with the host plant (Plenchette, 1982) and it has been suggested that the internal P status of the plant is the determining factor affecting mycorrhizal activity (Sanders, 1975 ; Menge et al., 1978). It has been proposed that the phosphorus inhibition of fungal colonization of roots is associated with membrane-mediated decreases in root exudation (Ratnayake et al., 1978). Whilst this may be true for extremely high levels of soluble phosphate fertilization, recent observations on external mycelium development (Bethlenfalvay et al., 1983 ; Jakobsen, 1983) and fungal phosphatases (Pearson and Read, 1975 ; Calleja et al. 1983 ; Gianinazzi-Pearson and Gianinazzi, 1983) in mycorrhizae indicate that increases in soluble phosphate supplies may directly disturb the physiology of the fungi before significantly affecting their ability to colonize the root tissues. There is evidence that some mycorrhizal fungi tolerate higher levels of soluble phosphate than others (Pons et al, 1984 ; Giltrap and Lewis, 1981). However, the effects of high soil and plant P on the processes of phosphate absorption, translocation and transfer to the host plant by mycorrhizal fungi have not been studied ; research into this is urgently needed if any advances are to be made in the understanding of how soil P levels affect mycorrhiza function.

There have been very few attempts to use physiological or biochemical markers to measure the effectiveness of mycorrhizae in phosphate nutrition. Succinate dehydrogenase (Ocampo and

Barea, 1982) and alkaline phosphatase activity (Gianinazzi-Pearson and Gianinazzi, 1978, 1983) have been used to measure fungal activity in VA mycorrhizae. The latter enzyme, which is associated with the fungal vacuoles, has been related to growth responses early in plant development. Polyphosphate accumulation, which seems to be an indication of the capacity of fungi to store phosphate, may also provide an interesting marker of mycorrhizal effectiveness in the phosphate nutrition of plants. It is evident that appropriate physiological or biochemical markers for mycorrhizal effectiveness in phosphate nutrition will only be found if much more research is devoted to the fundamental aspects of these symbiotic associations.

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MEMBRANE REGULATION OF MYCORRHIZAL SYMBIOSIS

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Mycorrhizal fungi apparently are obligately dependent on living plant roots for their supply of fixed carbon and other nutrients. Since neither vesicular-arbuscular (VAM) or ecto-mycorrhizal fungi penetrate root cell membranes, nutrients for growth and development of these fungi must first pass through the host plasmalemma. In the root cortex, cell metabolites are exuded into the apoplast between cortical cells and pass from the root surface as exudates. Root exudates should contain all the nutrients necessary to sustain the growth of mycorrhizal fungi (16).

Recently, it was proposed that VAM symbiosis is regulated by the amount of organic nutrients leaked from roots (4, 15). A fundamental relationship between phosphorus (P) content of root tissue, phospholipid content in root cell membranes, and changes in membrane permeability and exudation of organic compounds from roots was found (4). Based on the correlation between reduction in root exudation and inhibition of VAM colonization in P-sufficient roots, it was hypothesized that membrane-mediated decrease in leakage of root metabolites necessary to support the fungus during colonization is responsible for P inhibition of VAM formation (4). Although it has been suggested that greater VAM colonization in low P status root is correlated with increased content of soluble sugars in roots (7), the above-mentioned studies and others (5, 8) concluded that greater leakage of organic compounds is a more important determinant than concentration within root cells. Factors such as temperature, light intensity (5), photoperiod (7), flower bud development (8), ozone (11), and herbicides (17) have been used to alter the leakiness of root membranes or the supply of nutrients available for leakage as means of regulating VAM colonization without affecting P status of the plants. Apparently, changes in root exudation can affect VAM development independently of P nutrition.

Root exudation is greatest in the zone of elongation (12), the portion of root system that is most susceptible to VAM fungal colonization (2, 18). Carbon losses from roots in the form of root exudates appear to be sufficient to sustain the activities of VAM fungi (1, 19). Root metabolites could affect colonization at either the pre-penetration stage in the rhizosphere or at the post-penetration stage in the intercellular spaces, or at both stages. Root exudates from diverse plant types stimulate the germination and germ-tube development of Glomus epigaeum (3). Growth and development of hyphae are altered in close proximity to roots (13, 14). Also, a volatile attractant from roots may affect germ tube development and contact of roots by Gigaspora gigantea (10). The number of hyphal penetrations increases in P-deficient roots compared to those of higher P status (7). Taken together, these observations suggest that the activities of the mycorrhizal fungus are affected by root metabolites at least at the pre-penetration stage.

Following VAM colonization of P-deficient roots, improvement in P-nutrition results in decreased membrane permeability and a reduction in root exudation (4). Although spread of the VAM fungus slows after an initially rapid growth phase (2), colonization continues inspite of increased P nutrition. Reduced levels of exudation may be adequate to sustain mycorrhizal activity because of the greatly enhanced surface contact between host cell membranes and the plasmalemma of fungal arbuscules. Nevertheless P-control of membrane leakage of metabolites is a potential mechanism whereby the extent of fun gal growth in the root can be limited by host physiology.

VAM-induced decreases in root exudation as a result of improved P nutrition have been correlated with the reduction in soilborne disease (6). It is expected that mycorrhizae through their effect on root exudation, could alter the activities of microorganisms which respond to exudation in and around roots.

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MEMBRANE PHYSIOLOGY OF VESICULAR-ARBUSCULAR MYCORRHIZAE

By

S. Schwab

Research by Ratnayake et al. (1978) and Graham et al. (1981) showed a correlation between low phosphorus (P) levels in host plant tissue, high rates of leakage of metabolites from roots, and increased VAM formation. Unlike the studies by Jasper et al. (1979) and Same et al. (1983), Ratnayake and Graham concluded that the increase in leakage of metabolites was related to an increase in membrane permeability under P-deficient conditions, rather than to an increase in the concentration of metabolites in root cells. To further investigate the possible role of P-mediated changes in membrane permeability on VAM formation, studies were done to determine: (1) which stage(s) of the colonization process are affected by P nutrition; (2) if P nutrition is associated with qualitative as well as quantitative changes in metabolites leaked from the root; and, (3) if soluble metabolites contained within root cells are an accurate reflection of the amount and kind of metabolites which cross the host plasma membrane and are hence available to the fungus.

The effect of P nutrition on stages of VAM formation was compared in sudangrass grown in high and low P soil. Measurements of spore germination were made at two and four weeks, and measurements of number of colonization points, mean length of each infection, percent of root length colonized, total length of root colonized, morphology of arbuscules, and extent of external hyphal growth were made at 10 day intervals from 15 to 45 days after inoculation. Quantitative and qualitative measurements of the composition of soluble metabolites leaked from root cells and metabolites extracted from root tissue were made in noninoculated sudangrass six weeks after plant germination.

Neither spore germination, initial contact and penetration into the root, nor morphology of VAM were affected by P-nutrition. Decreased percent and decreased total length of root colonized in plants with high tissue P seemed to be related to post-penetration phenomena, particularly growth of external hyphae which may act as sources of secondary infection (Schwab, et al. 1983a). P nutrition had little qualitative effect on leakage of metabolites from roots, but it did reduce the quantity of metabolites leaked from roots (Schwab, et al. 1983b). The effect of P-nutrition on quantity and composition of soluble root extracts did not parallel its effect on the quantity or composition of metabolites leaked from roots. Because VAM do not normally disrupt the host plasma membrane, the apparent ability of the membrane to regulate the quantity and compositon of metabolites that enter the apoplast may be an important factor modifying colonization of the root by VAM fungi.

Calculations of the rate of fungal growth in roots with the rate of leakage of metabolites suggest that no single class of compounds could act as the sole substrate for VAM fungi, unless the endophyte is capable of inducing a higher rate of transport across the plasma membrane than occurs in noncolonized roots (Schwab, et al. 1983b). Comparisons of the properties of plasma membranes of cortical cells of colonized and non-colonized roots would therefore be of considerable interest. Isolation of protoplasts from colonized and noncolonized roots should allow for comparisons of membrane lipid and protein content, transport kinetics of both phosphorus and carbon, and operation of ATPase mediated active transport.

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PHYTOHORMONE ACTION: AN INTEGRATIVE APPROACH TO UNDERSTANDING DIVERSE MYCORRHIZAL RESPONSES

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Plants must overcome stress to survive and reproduce. Because they are immobile and, unlike animals, remain subject to the stresses at the site of establishment, they have evolved growth and developmental mechanisms which are highly plastic; they tolerate high stress levels and avoid stress through time. Growth and development are regulated both genetically and environmentally. Plant hormones regulate almost all aspects of plant growth and development, particularly those under environmental control (Wareing and Phillips 1981). Mycorrhizae are mutualistic associations in which the two symbionts, plant and fungus, have co-evolved, possibly since the time of the earliest land plants. Presumably, mycorrhizae evolved in response to stress: plants have limited capacities for nutrient and water uptake, and the fungus has limited carbon availability. As these two groups coevolved, both genetic and environmental cues regulated the association. If mycorrhizae alleviate stress in plants, then the hormonal status must change to provide a balanced response to the environment by that plant.

In a mycorrhiza-forming plant growing under conditions where a nutrient or water is limiting and inoculum is present, a series of steps characterizes the infection process which, in turn, alleviates that stress (Figure 1). Plant hormones have been shown to be important in the infection process by influencing both spore germination (Allen unpublished observations) and mycorrhizal formation (e.g. Slankis 1973, Azcón et al. 1978) After mycorrhizal establishment, hormonal changes in the plant in conjunction with the improved nutrient and water status at least partially alleviate the imposed stress (Allen et al. 1980, 1982). However, the exact nature of how the plant aquires those hormonal changes and uses the hormones to alter nutrient, carbohydrate, and water allocations remains unknown. At present we know that mycorrhizal fungi can produce hormones, that some morphological and anatomical changes in plants with mycorrhizae resemble changes associated with altered hormone balances, and that hormonal changes in the host plants are associated with mycorrhizal infection. However, we don't know how, or even if, hormones are directly transported from fungus to plant or how mycorrhiza-altered hormonal balances regulate plant physiological and developmental processes to alleviate stress.

Several species of ectomycorrhizal fungi are known to produce a range of phytohormones (e.g. Slankis 1973, Crafts and Miller 1974, Harley and Smith 1983), and there is one published demonstration of hormone production by a VAM fungus (Barea and Azcon-Aguilar 1982). However, there is substantial variability in production both among isolates (Ek et al. 1983) and growth conditions (Rudawska 1982). There are also several reports of morphological changes in host plants which resemble changes produced by adding hormones to a plant growth medium (e.g. Hadley 1970, Slankis 1973). MacDougal and Dufrenoy (1944) showed higher auxin concentrations in mycorrhiza-infected root cells than adjacent cells, but their technique is no longer considered adequate. We (Allen 1980, Allen et al. 1980, 1982) demonstrated that the balance of several hormones was changed with mycorrhizal infection in a VAM system. These data demonstrate that mycorrhizal formation alters plant hormone balance but do not demonstrate that fungal-produced hormones are transported into the host plant and remain active. Hormones can be taken up by plants from an external medium (e.g. Wareing 1982). However, roots may contain surface enzymes capable of breaking down external hormones (A. Anderson, personal communication). Testing for direct transport of a hormone from fungus to plant is probably the most important immediate need for further research of mycorrhiza-hormone interactions.

Mycorrhizal establishment results in a wide range of physiological adjustments by the plant to both the symbiont and the external environment. Many are obvious, such as the transport of nutrients and water from soil to plant via the fungal hyphae. However, many of the physiological adaptations of a plant to mycorrhizal symbiosis are subtle and also represent processes regulated by hormone action (Figure 2). For example, mycorrhizal infection often increases plant growth and seed production with water or phosphorus (P) stress. Mycorrhizal fungi can take up inorganic P and water and transport them

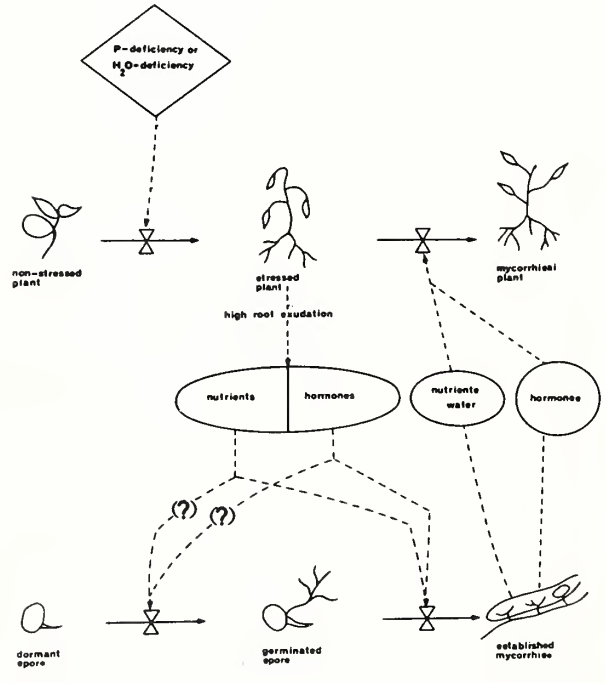

Figure 1. Potential role of hormones in the establishment of a mycorrhizal association.

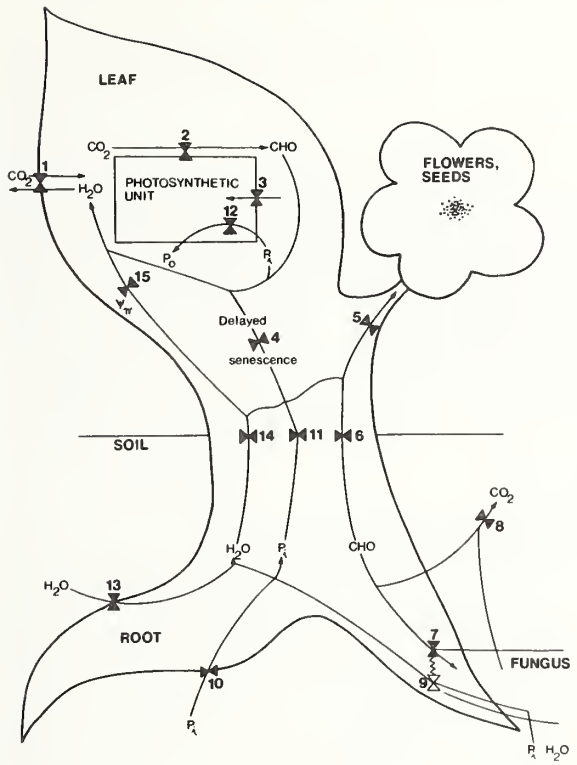

Figure 2. Comparison of physiological changes in the host plant associated with mycorrhizal formation ⋈ and those changes which are known to be regulated by both hormone action and mycorrhizae ⋈. P_i=inorganic phosphorus, P_o=organic phosphorus. See text for explanation of valves which regulate flows.

into the host plant (Figure 2, regulation valve 9). However, for those increased nutrients and water to influence CO_2 fixation and carbohydate allocation, a whole range of physiological changes, regulated by hormone action, must occur simultaneously. The nutrients and water must not only be taken up in the roots (valves 9, 10, 13) but must be moved to the leaves (11, 14). Stomates must open wider or remain open longer (1) in response to altered ψ_π (15) and/or delayed senescence (4). There must be an increase in the photosynthetic machinery (3), increasing utilization of P as high energy intermediates (12), and fixing the increased CO_2 taken up by the leaves (2). If the resulting carbohydrates are to be effective, then allocation to roots, fungus, reproductive structures, and respiration (5, 6, 7, 8) must be tightly regulated. All of these changes have been documented as mycorrhizal responses (see Allen 1984, Harley and Smith 1983 for reviews), and they are also controlled by hormones (e.g. Wareing and Phillips 1981, Wareing 1982), with the exception of valve number 9. There is no documented evidence for direct influence of hormones on uptake of water and nutrients by mycorrhizae.

Different isolates of mycorrhizal fungi can result in altered physiology of a given host plant (e.g. Harley and Smith 1983). Some of these changes are not easily ascribed to simple uptake of nutrients or water (e.g. Allen and Boosalis 1983, Stahl and Smith 1984). Different mycorrhizal fungal isolates or environmental conditions can change types and levels of hormones produced by the symbionts (e.g. Crafts and Miller 1974, Rudawska 1982, Ek et al. 1984). Thus, changes in fungal isolates or environmental conditions might stimulate hormonal changes in the mycorrhizal symbiosis resulting in observed variable plant responses.

Relative rates of all of the described physiological processes (Figure 2) can be readily manipulated by small changes in the balances of these hormones. By stimulating minor changes in plant hormone balance coupled with direct transport of nutrients and water, mycorrhizal associations may cause much of the plasticity of plants necessary to cope with stress. It is only through understanding those integrative actions, nutrients, water, and hormones, that a comprehensive understanding of mycorrhizal associations will be gained. Moreover, the applied uses of mycorrhizae, whether for developing rapidly establishing inoculum or for developing better strains, depends on a holistic understanding of the mechanisms by which mycorrhizal associations affect plant growth and development.

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VA MYCORRHIZAS: PLANT AND FUNGAL WATER RELATIONS

By

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Introduction

Research on the water relations and drought tolerance of VA mycorrhizal systems has increased dramatically in recent years. At least two reviews on this subject ahve been published (1,2). Studies on the water relations of mycorrhizal fungal growth and development and on the process of root colonization are few in number, however, interest in these areas is also increasing. We will attempt to evaluate some of the published data in these areas and to suggest additional experimental approaches.

Well Watered Conditions

Safir et al. (3) were the first to report that VA mycorrhizal root colonization altered plant water relations. They found that, under well watered conditions and low soil nutrient levels, mycorrhizal soybean plants had higher hydraulic conductivities than did nonmycorrhizal controls. The increased conductivity occurred when mycorrhizal plants were larger than the nonmycorrhizal controls. Four hypothesis were presumed to explain the increased hydraulic conductivities of mycorrhizal plants. First, the increased surface area provided by the external hyphae might increase water absorption. Second, the hyphae within the root might provide a low resistance pathway for water movement. Third, increased plant nutrient uptake caused by the fungal hyphae could increase the hydraulic conductivities of the roots. Fourth, mycorrhizal root colonization could increase the size of the root system and make it more conductive. The last hypothesis was unlikely since the root systems were not different in size. Safir et al. (4) found that most of the increased hydraulic conductivity of mycorrhizal plants was associated with the roots. They also found that the addition of nutrients to the soil at the time of seeding increased the hydraulic conductivities of the nonmycorrhizal plants to a level similar to that of mycorrhizal plants. This supported the hypothesis that the increased conductivities of mycorrhizal plants was related to improved nutritional status. The possibility that the fungal hyphae within the root could provide a low resistance pathway within the root was tested by measuring hydraulic conductivities after addition of the fungicide pentachloronitrobenzene to the roots. The fungicide did not alter the hydraulic conductivities of either mycorrhizal or nonmycorrhizal plants. Since this fungicide is known to reduce mycorrhizal enhancement of nutrient uptake, it should have altered the conductivities of mycorrhizal plants if the internal hyphae are significantly involved in water transport. Further experiments should be conducted to evaluate this possibility.

Levy and Krukun (5) studied the water relations of similar sized mycorrhizal and nonmycorrhizal 8-month-old citrus trees before, during and after a water stress period. During stress development leaf water potentials, stomatal resistances and calculated transpiration rates of mycorrhizal and nonmycorrhizal trees were not significantly different. These results indirectly support the results of the high fertilization experiments of Safir et al. (4). Levy and Krukun (5) also presented data showing that mycorrhizal citrus trees, during recovery from water stress, had higher transpiration rates and stomatal conductances than nonmycorrhizal controls. They postulated an involvement of altered hormone balances in these differences.

Hardie and Leyton (6) found that mycorrhizal clover roots had higher hydraulic conductivities than nonmycorrhizal clover roots on a per unit root length basis. They postulated the involvement of the fungal hyphae in transporting water to the roots. The mycorrhizal plants had higher leaf areas, fresh and dry weights and higher phosphorus (P) levels than did the nonmycorrhizal controls. Increased soil P levels resulted in increased nonmycorrhizal root conductivities. Mycorrhizal plants also had higher transpiration rates and lower leaf diffusion resistances than nonmycorrhizal plants. In addition, the mycorrhizal clover plants recovered from water stress faster than nonmycorrhizal plants. The faster recovery from water stress of mycorrhizal soybean plants was previously demonstrated by Safir et al. (3).

Allen et al. (7) studied the water relations of 5-month-old non-fertilized mycorrhizal and nonmycorrhizal Boutaloua gracilis plants. Mycorrhizal plants had lower leaf diffusion resistances, higher transpiration rates and higher hydraulic conductivities than nonmycorrhizal plants at several soil moisture levels. Root and leaf phosphorus levels were higher in the mycorrhizal plants. In a later study by Allen (8) mycorrhizal B. gracilis had higher hydraulic conductivities, higher transpiration rates and lower leaf diffusion resistances at similar leaf water potentials. Based on an analysis of hyphal entry points per unit length of root they postulated that the increased water uptake by mycorrhizal plants could be attributed to direct fungal uptake and transport. We feel that although this theory is possible, several assumptions made in the analysis may not be warranted. First, they assumed that the rate of water uptake per unit root surface area is similar between mycorrhizal and nonmycorrhizal plants. This would not be true if phosphorus levels were higher in mycorrhizal roots. For example, Hardie and Leyton (6) demonstrated that increased root P levels were associated with increased root hydraulic conductivities. Also, it is likely that the mycorrhizal B. gracilis plants used by Allen et al. (7) had increased phosphorus nutrition since the plants were more than 2 months old and were not fertilized. Second, Allen et al. (7) used water uptake rates in their calculations as reported for Phycomyces blakesleeanu (9) which were under vapor driving gradients of between -7.0 and -15.7 MPa for uptake rates of 131 n l hr^{-1}. The driving gradient between the root cortex and the soil is likely to be a great deal less than -7.0 MPa. Third, experiments in our laboratory have failed to demonstrate water uptake by hyphae over a 24 hr period that was sufficient to alter leaf water potentials of soybeans under

non-transpirational conditions. In addition, using tritiated water, we have not been able to demonstrate appreciable hyphal transport of water to mycorrhizal onion plants. It is more than likely, however, that small amounts of water are transported from the hyphae to the roots (10).

Nelsen and Safir (11) studied the water relations of mycorrhizal and nonmycorrhizal onion plants under well-watered conditions. At soil P levels of 10 ppm, mycorrhizal plants were larger, had higher transpiration rates, lower leaf water potentials and higher hydraulic conductivities than nonmycorrhizal controls. When nonmycorrhizal plants were grown at soil P levels of 60 ppm, their growth was similar to the mycorrhizal plants grown at 10 ppm soil P. Also, there were no differences in water relations between the fertilized (60 ppm) nonmycorrhizal plants and mycorrhizal plants. Nelsen and Safir suggested that under conditions of high water and phosphorus availability mycorrhizal infection would not have major effects on plant water relationships. Under conditions of low P availability, the increased P nutrition and hydraulic conductivity of mycorrhizal plants should result in higher leaf water potentials and lower diffusion resistances.

Drought Stress Conditions

Nelsen and Safir (12) studied the effects of mycorrhizal root colonization on onion plants exposed to 7 cycles of water stress over an 8 week period. Nonmycorrhizal plants were grown in soils containing high soil P levels (36 ppm) and mycorrhizal plants were grown in low P soils (6 ppm). At these soil P levels mycorrhizal and nonmycorrhizal plants were similar in size when grown under well-watered conditions. At the end of the stress period the mycorrhizal plants were 4 times larger than the nonmycorrhizal plants, which had almost ceased to grow after the first drought cycle. Although water stress reduced the growth of both mycorrhizal and nonmycorrhizal plants only the nonmycorrhizal plants were deficient in phosphorus. Since mycorrhizal and nonmycorrhizal leaf water potentials were similar and since soil P levels did not change during the experiment, it was suggested that nonmycorrhizal plants were not capable of absorbing adequate levels of P for growth during severe drought. Phosphorus mobility decreases dramatically in dry soils. These results suggest that the benefits of mycorrhizal root colonization are likely to be greater in dry soils. This contention is supported by a field study of Bolgiano et al. (13). Onion plants were grown under 2 watering regimes and at 3 soil P levels. Soil P levels above 15 μg cm^{-3} greatly reduced mycorrhizal root colonization in irrigated plots. In nonirrigated plots root colonization was reduced only at soil P levels above 30 μg cm^{-3}.

Earlier greenhouse studies of Sieverding (14) also strongly suggest that mycorrhizal root colonization will be more beneficial under limited water supply conditions. These increased benefits were largely attributed to the increased P absorbing power of mycorrhizal plants under dry conditions.

Levy et al. (15) exposed citrus seedlings to 3 cycles of water stress. The stressed mycorrhizal plants had greater root length and transpiration roots, and lower leaf water potentials than nonmycorrhizal controls. The authors pointed out that the slightly larger mycorrhizal plants depleted the soil water supply faster than the nonmycorrhizal plants causing more severe water stress in the mycorrhizal plants. The root hydraulic conductivities of water stressed plants were lower than those of non-stressed plants and mycorrhizal infection further reduced root conductivity. The lowered conductivity of mycorrhizal roots may have been caused by the greater water stress to which mycorrhizal plants were exposed. Tissue P levels were not dificient for either mycorrhizal or nonmycorrhizal plants.

Allen and Boosalis (16) compared the effects of two mycorrhizal fungi on the water relations and drought tolerance of greenhouse grown wheat. Stomatal conductances were higher for mycorrhizal plants than for nonmycorrhizal plants under both wet and dry conditions and stomatal closure of mycorrhizal plant leaves occurred at lower leaf water potentials and after greater desiccation than for leaves from nonmycorrhizal plants. Dry weights of Glomus fasciculatum infected and nonmycorrhizal plants were similar under both wet and dry conditions while Glomus mosseae infected plants were smaller than nonmycorrhizal plants. G. fasciculatum infected plants had lower osmotic potentials and higher turgor under stress conditions than nonmycorrhizal controls, which would appear to increase drought tolerance of the infected plants. These results are very interesting and research to study the mechanisms involved in the apparent drought tolerance of G. fasciculatus infected plants is necessary. It would be useful to know what the tissue P levels, and the leaf areas were for the above experiments. Increased hydration or fresh/dry weight ratios have been demonstrated for mycorrhizal onion plants under some conditions (17). Also, mycorrhizal citrus seedlings have higher fresh/dry weight ratios than nonmycorrhizal seedlings when mycorrhizal leaf P concentrations are higher than those of nonmycorrhizal plants. As the P concentrations of nonmycorrhizal citrus leaves increase their fresh/dry weight ratios increase and approach the ratio levels of mycorrhizal plants (C.R. Johnson, personal communication). The need for additional research on the water relations of mycorrhizal wheat is further emphasized by the fact that Glomus etunicatum infection has been shown to slightly decrease the yield of wheat plants under both wet and dry conditions (18).

Fungal Growth and Development

Redhead (19) found that the amount of soil water that was optimal for plant growth resulted in the maximum production of fungal spores. This suggests that fungal sporulation is dependent on plant size or plant carbohydrate availability. In support of this idea, Nelsen and Safir (12) found that spore production of Glomus etunicatum was reduced by 61% and 51% and onion fresh weights reduced by 68% and 67% by drought stress at low and high soil P levels, respectively.

Reid and Bowen (20) showed that the number of infections per root length of barrel medic doubled when the soil matric potential was decreased from -0.1 to -0.19 MPa and was nearly 40 times greater at -0.19 than at -1.4 MPa. Sieverding showed

that infection of sorghum and crucita by Glomus macrocarpus was enhanced in dry soils. He postulated that lowered nutrient availability, especially P, in dry soils was responsible for increased mycorrhizal infection and growth promotion. Bolgiano et al. (13) confirmed this hypothesis by showing that mycorrhizal infection of onion in the field occurred at higher soil P levels when soil water status was decreased. Similarly, Levy et al. found that decreased irrigation increased mycorrhizal infection of citrus in the field (21). The numbers of papers are few concerning the water relations of mycorrhizal fungal growth and root colonization. It is likely that the water-nutrient-temperature requirements for each fungus and fungus-host combination will be different. More critical studies are needed.

Conclusions

There is little question that mycorrhizal root colonization can alter plant water relations under both wet and dry conditions. This is not surprising considering that large changes in plant growth that can result from root colonization At this time we can only speculate about the reasons for the altered water relations of mycorrhizal plants.

We believe, however, that most of the reported effects of mycorrhizal root colonization on plant water relationships are likely to be related to the improved nutrition (particularly P) of mycorrhizal plants.

Unfortunately, in most cases where plant nutrition is studied along with water relation parameters, the phosphorus concentration of nonmycorrhizal plants are at or near deficient levels. In one case where changes in transpirational behavior of mycorrhizal plants did not appear to be related to tissue P levels, there were differences in water stress between mycorrhizal and nonmycorrhizal plants (15). In another study (16) which strongly suggested that mycorrhizal and nonmycorrhizal plants of similar dry weights were different in terms of drought tolerance, information concerning plant P nutrition and leaf area or fresh/dry weight ratios was not presented. An analysis of fresh/dry weight ratios, leaf areas, and phosphorus nutrition must be included in future studies of the comparative water relations of mycorrhizal and nonmycorrhizal plants. In order for differences between the water relations of mycorrhizal and nonmycorrhizal plants to be explained by mechanisms other than nutrition the mycorrhizal and nonmycorrhizal plants should be similar in size, fresh/dry weight ratios, P nutrition and stage of development.

Furthermore, short term differences in water use between mycorrhizal and nonmycorrhizal plants are not necessarily related to drought tolerance. It would thus be desirable to conduct long term replicated experiments under simulated field or field conditions with water availability and evaporative demand conditions precisely controlled. In cases where P levels of nonmycorrhizal controls are difficult to elevate to the levels of mycorrhizal plants, foliar feeding of phosphorus should be considered. The possible involvement of VA mycorrhizal root colonization in host drought tolerance is exciting, especially, since these effects may not be alleviated by soil fertilizer applications.

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Carbon Flow in Mycorrhizal Plant Associations

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Keywords: Glomus, Rhizobium, Azospirillum, photosynthesis, C allocation, N_2 fixation, soybean sorghum.

Introduction

Mycorrhizal research has been characterized by the recognition of the wide diversity of fungal-plant associations. This has resulted in a great deal of parallel research that has extended our range of understanding but provided few major new insights. The literature shows a great deal of similarity among the processes affected by these associations. Concentration on the processes and their controls should make it possible to increase our understanding while also providing management alternatives for these associations in the diversity of conditions that occur in nature.

The major nutritional effects of symbiosis center around the carbon (C) and phosphorus (P) and nitrogen (N) interactions in two and sometimes three partner associations. The significance of C supply from the host plant was recognized by Franck at the initiation of mycorrhizal research. Symbiosis would not exist if the individual participants could not adapt to the needs of the whole. It is useful to consider the symbiotic system as a balance of flows, in which the plant supplies the C while the microbial partners supply inorganic nutrients. In this discussion, we attempt to summarize the present status of understanding while stressing unifying concepts in the field of C allocation from the plant to microbial symbionts.

Carbon Flow

The movement of tracer C from plant to fungus was shown in ectomycorrhizae in 1957 (Melin and Nilsson) and in VAM (Ho and Trappe) in 1973. In VAM the movement of C from the host to the fungus is presumed to occur in the arbuscule. Possible mechanisms for C transfer include: active uptake of hexoses from the arbuscule, disorganization of cell wall development and active hydrolysis of normal cell wall components (Harley and Smith, 1983). In VAM, the conversion of plant C to glycolipids may constitute a mechanism for the accumulation of substrate by the fungus. In ectomycorrhizae, the production of carbohydrates such as trehalose and mannitol not readily metabolized by the plant cells may have a similar function. The specific mode of transfer and compounds involved however, are not known and there may be no necessity for the fungus to exhibit a mechanism other than to provide a concentration gradient for mass flow.

Carbon Flux to Symbionts

Estimates of VAM biomass range from 3 to 14% of the host root mass with the lowest estimate being found in the fibrous root system of sorghum (Table 1). Similar values apply for the available measurements of ectomycorrhizae when the total beneath ground root system is considered. However as much as 40% of the fine roots of trees can be mycorrhizal; significant C flow is said to occur through mycorrhizal fungi, sclerotia and sporocarp turnover within the forest system (Fogel and Hunt, 1979; Vogt et al., 1982).

Table 1. Biomass of mycorrhizae in the roots of symbiotic associations.

Fungus	Host	% Root Wt	Method	% Infection	Ref.
G. fasciculatum	Centrosema pubescens	14	chitin	95	1
G. mosseae	Centrosema pubescens	7	chitin	100	1
A. laevis	Centrosema pubescens	5	chitin	95	1
G. mosseae	Vicia faba	6	microscopy	60	2
G. mosseae	Allium porrum	10	estimate	65	3
G. fasciculatum	Glycine max	16	chitin	70	4
G. fasciculatum	Sorghum bicolor	3	chitin	50	5
Ectomycorrhizae	Abies amabilis				
	23 yr	12	sampling		6
	180 yr	3	+ estimate for mantle %		6
	Pseudotsuga menziesii	15	microscopy + harvest		7

(1) Hepper, 1977; (2) Kucey & Paul, 1982; (3) Snellgrove et al, 1982
(4) Bethlenfalvay, 1981; (5) Harris et al, 1984; (6) Vogt et al, 1982
(7) Fogel & Hunt, 1979

The attempts to directly measure the C cost of mycorrhizal symbiosis have used pulse chase labelling with $^{14}CO_2$ to measure the flux and distribution of photosynthate in plants with or without mycorrhizal fungi. The possible alteration of photosynthetic rates to compensate for differences in C allocation and the needs of other symbionts such as N_2 fixing organisms must be considered in the interpretation of such data. The increased beneath ground C demands can lead to negative growth effects from the consumption by the microbial partner of photosynthetically derived C if concomitant positive effects on nutrient uptake and plant growth do not occur.

Available information shows 4% to 17% of the plant C flux can be attributed to the effects of mycorrhizal infection (Table 2). Pulse labelling studies of C-flow in the plant-rhizobium-VAM association have confirmed the possibility of photosynthetic compensation for the cost of both the microbial partners in plants adjusted to equal size by N or P additions to non-infected plants (Kucey and Paul, 1981). Data for 6-week-old soybeans (Figure 1) shows that 60% of the photosynthate was retained above ground. In this association, VAM fungal biomass and nodular tissue each accounted for only 1-2% of the photosynthate, but their individual respiration each accounted for 11-13% of the fixed C. Together this represented 70% of the beneath ground respiration. The data in Figure 1 indicate a very low growth yield (0.1-0.15) for both nodules and mycorrhizal fungus. This is not surprising for the nodule but is difficult to explain for VAM fungi. Possible explanations include:

1) The additional respiration in mycorrhizal roots is in part due to increased plant root respiration (Cox and Tinker, 1976).

2) Under estimation of extraradical fungal biomass.

3) ^{14}C contents of fungal tissue are based on specific activity of external hyphae which underestimate intraradical ^{14}C incorporation.

4) Mycorrhizal symbiosis is based on high C respiration for nutrient uptake and translocation with some limit on fungal growth. High growth efficiencies would tie-up nutrients in the hyphae. This in turn may explain why mycorrhizal fungi are so dependent on plant supplies of C.

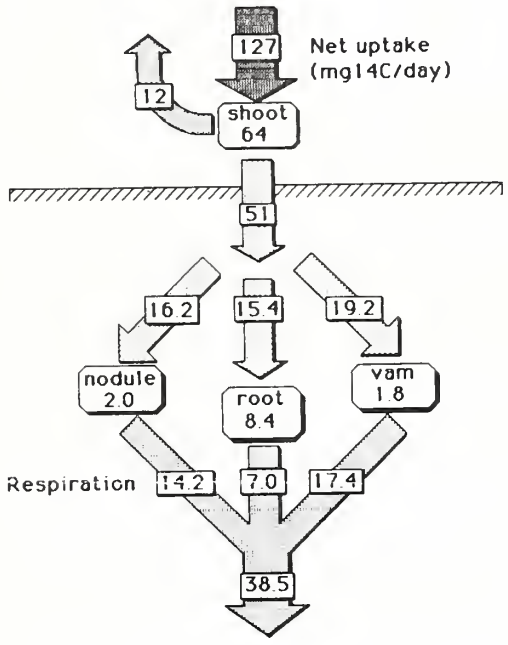

Figure 1. C-allocation in Soybean-Rhizobium-VAM associations (6 weeks).

Table 2. Effects of mycorrhizae on C allocation and photosynthesis.

		% Control		
Fungus	Host	C-allocation to symbiont	C-uptake (net photosyn.)	Ref.
G. mosseae	Vicia faba (nodulated)	+10	+21	1
G. mosseae	Vicia faba (nodulated)	+5 +6	+8 +20	2
G. fasciculatum	Glycine max (nodulated)	+14	+20	3
G. fasciculatum	Sorghum bicolor	+4	-21	4
G. mosseae	Allium porrum	+4 +17	-2 +29	5
P. tinctorus	Pinus	+16	+17	6

(1) Pang & Paul, 1980; (2) Kucey & Paul, 1982; (3) Harris et al, 1984; (4) Harris et al, 1984; (5) Snellgrove et al, 1982; (6) Reid et al, 1982.

It has also been noted that rhizobium appears to get first call for the C in the presence of both rhizobium and the fungus VAM (Harris and Paul, 1983). This is not entirely unexpected in light of the high C demand of the N_2 fixing bacteroids and the close association between the nodules and the plant vascular system.

Plant Responses to Microbial Symbionts

Table 2 indicates a general capability of the plant to exploit otherwise under-utilized photosynthetic capacity to compensate for the needs of the symbiotic partner in all of the systems except sorghum and leek. The C-4 sorghum plant with a high density of fibrous roots supported only a small fungal biomass and showed a large negative photosynthetic response. This could be attributable to the differences in growth between fertilized controls and the plants dependent on symbiotic associations indicating the inability of the microbial partners to supply adequate levels of nutrients under these conditions.

In our work showing photosynthetic enhancement, Rhizobium alone had little effect on specific leaf area, leaf hydration, the concentrations of N and P or the storage of carbohydrates (Table 3). The addition of VAM to the association resulted in increased leaf area without effects on leaf water content. Foliar N and P concentrations increased by 22% and 78% while the amount of starch in the leaves was halved. The two mechanisms most commonly used to explain C fixation rates and allocation patterns in mycorrhizal plants involve aspects of P or N nutrition and source/sink regulation. The nutritional argument purports that mycorrhizae enhance or diminish element(s) within the plant. This in turn facilitates or inhibits photosynthesis or C-transport (Figure 2). Phosphorus affects leaf C metabolism by affecting leaf expansion (Natr, 1975). In turn, photosynthetic rates are affected by: (a) levels of RUBP (Heldt et al, 1978), (b) ATP/ADP ratios (Robinson and Walker, 1979) and (c) CO_2 resistance and regulation of stomates. P levels also affect phosphate translocator (TPT) activity operating across the chloroplast membrane (Heldt and Rapley, 1970) and may control partitioning between starch and sucrose in the leaf mesophyll cell (Priess, 1984).

Table 3. Leaf characteristic in Soybean-Rhizobium-VAM associations*

Symbionts	None	Rhiz.	R+VAM
Specific leaf area (dm2/g)	164	179	204
H_2O%	73.5	73.7	75.6
Net Fixation			
mgC/g/h	2.05	2.72	3.26
mgC/dm2/h	1.26	1.58	1.60
Sugars %	9.6	10.5	11.8
Starch %	16.0	16.8	7.7
Total N %	2.28	2.10	2.78
Total P %	0.09	0.09	0.16

*Harris, Pacovsky & Paul (in preparation)

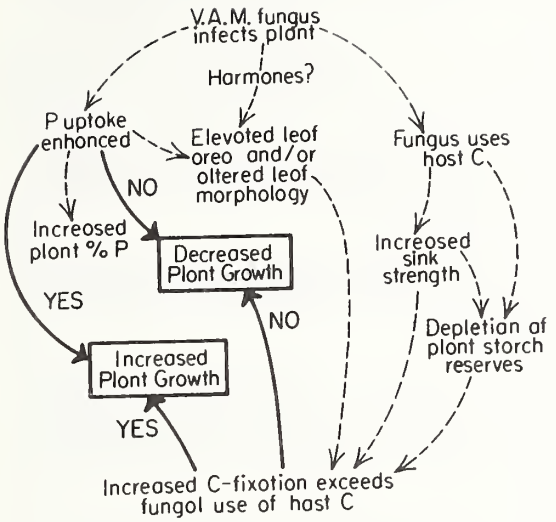

Figure 2. C-P-N interactions in mycorrhizal associations

The most cited effects of N nutrition on plant leaves include effects on chlorophyll contents, RUBP carboxylase activities and changes in leaf resistances to CO_2 diffusion. The ramifications of enhanced N concentrations in the shoot and mycorrhizal uptake of other elements significant to plant growth must also be considered. The enhancement of the C allocation to mycorrhizal roots by increased sucrose concentration gradient has been be attributed to: (a) hormones of fungal origin (Slankis, 1973), (b) alteration of plant-synthesized hormone levels (Allen et al, 1982), (c) elevated root invertase activity or impaired plant cell wall synthesis (Harley and Smith, 1983), (d) a generally elevated demand by the mycorrhiza for sucrose (Herold, 1980).

The translation of increased sink demand into elevated photosynthetic rates requires consideration of the biochemistry of leaf mesophyll cells and the effects of intermediates such as starch, sucrose, and triose-P. A lowering of sink demand could result in starch accumulation in the chloroplast and a physical distortion of the photosynthetic apparatus (Thorne and Koller, 1974). Elevated leaf sucrose levels affect enzymes such as sucrose-P synthetase and sucrose-P phosphatase (Salerno and Pontis, 1980); which could result in elevated triose-P levels and diminished P_i concentrations in the chloroplast. The triose-P/P_i ratio is looked upon as a critical parameter affecting the various enzyme activities involved in leaf C partitioning (Priess, 1984).

The Association in the Field

Mycorrhizal and rhizobial effects are well recognized symbiotic parameters. Less recognized are the interactions with organisms such as Azospirillum. VAM generally increases N_2 fixation by rhizobia, because of increased P levels. The effect of VAM on the Azospirillum (Harris et al, 1984) appears to be competitive in that the addition of VAM to the sorghum Azospirillum association results in the lowering of N_2 fixation rates. The observation of competitive interaction between mycorrhizal fungi and Azospirillum where both organisms occupy the cortex and the C utilized by the microorganisms was not compensated for by increased photosynthesis could explain the great variability in N_2 fixation measurements found for this association.

Improved management of symbiotic associations will require optimization of the contribution of all the partners involved. Genetic engineering will probably first be successfully applied to the microorganisms. There is major scope for selection of plant characteristics as well as genetic engineering to enhance soil nutrient dynamics and stability. Some possible plant alterations to achieve greater microbiological interactions are shown in Figure 3. Roots that decompose easily and have no residue are desirable under certain cultural conditions. Other areas require leaves that provide a persistent mulch for water infiltration and prevention of soil erosion. The proportion of lignin and tannin would therefore have to be selected (or engineered) while high photosynthetic activity was maintained. An extensive network of fibrous roots near the soil surface would provide better water penetration, erosion resistance and nutrient uptake and could minimize the need for mycorrhizal fungi. So the advantages of plants without mycorrhiza also should receive further investigation.

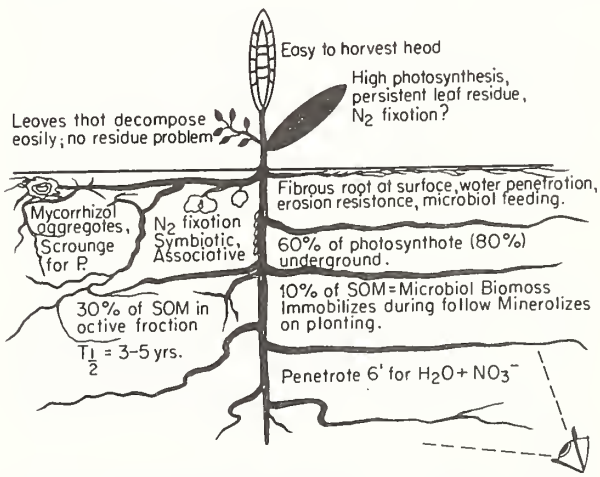

Figure 3. The perfect plant from a microbiologists viewpoint.

Mycorrhizal effects on aggregation are intertwined with C availability and polysaccharide production. These could be greatly enhanced by research projects designed with specific objectives in mind rather than mere observation of what occurs in nature. N_2 fixation by symbiotic associations has been adequately discussed elsewhere; it continues to have great potential as long as the role of the

overall plant association, including the mycorrhizal fungi, is recognized. Although leaf N_2 fixation should continue to be considered there is still a great deal of undeveloped potential in the Rhizobium and Azospirillum type associations.

The soil microbial biomass contains nutrient levels high enough to be a major source-sink for N, P and S. Management of this biomass such that immobilization occurs during fallow periods and mineralization during plant growth has been suggested as a way of increasing soil and fertilizer use efficiency (Paul and Voroney, 1984). The biomass normally constitutes 4-7% of the soil N. Raising this level to approximately 10% while also producing a larger proportion of active soil organic matter such that nutrient release would be higher on an extended basis, would require extensive beneath ground C-allocation. Figure 1 shows 40% beneath ground allocation. We often find that legumes with both mycorrhizal and rhizobial symbionts allocate up to 60% of their C to underground needs. Production of a soil with a raised active organic matter and microbial biomass content, in addition to providing the C for mycorrhizal aggregation, and P uptake, as well as for bacterial N_2 fixation, would lead to extensive demands on photosynthate. A possible movement of 80% of the photosynthate underground seems very high, but the possibility of developing plants with such a capacity while at the same time having very high yield potential is worth investigating.

Much of the work on P, C and hormone interactions with photosynthesis has been done with isolated leaf components or leaf discs. Extrapolation to the whole plant has to be done with care. There is a major need for an understanding of the processes involved in the whole plant with special emphasis on the controls in the multi-organism association. It is hoped that teams including molecular biologists, physiologists and soil microbiologists will rise to the challenge of understanding and management of the interaction in these associations.

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MYCORRHIZAL SYSTEMS AND "OTHER" ELEMENTS, ESPECIALLY MICRONUTRIENTS

By

J. L. Young and W. M. Jarrell

Introduction

As apparent in preceding segments of these NACOM sessions, prevailing opinion holds that the effectiveness of mycorrhizal fungi is dominantly, if not exclusively, a direct or indirect effect of how the symbiont affects the plant P status. Central focus remains on P relationships but periodically spills over to consideration of one to many other elements including macro-/micro-nutrients and toxic/heavy metals. Definitive data or literature on "other elements" will build slowly, as the varied results, interpretations, and opinions at these meetings attest. That is understandable in view of the numerous fungal species and strains, host types and cultivars, critical environmental factors, possible combinations thereof and all the compounding interactions that need to be considered (quite analogous to the complexities encountered in other microbial ecology studies, e.g., Babich and Stotzky, 1983a and 1983b).

Overview

For perspective on what is known to the present, readers may profitably consult recent documented reviews which became available just prior to the 6th NACOM gathering. Portions of Harley and Smith's 1983 Mycorrhizal Symbiosis are pertinent, as are several chapters in Metals and Micronutrients: Uptake and Utilization by Plants (Robb and Pierpoint, 1983). Chapter 2 by Tinker and Gildon entitled "Mycorrhizal Fungi and Ion Uptake" is of particular interest. But several other related chapters bear directly on the topic and merit more than casual attention (e.g., Chapter 4, "Adaptation to Toxic Metals"; Chapter 6, "Boron and Membrane Function in Plants"; and Chapter 14, "A Perspective of Mineral Nutrition: Essential and Functional Metals in Plants"). Yet other chapters in VA Mycorrhiza, a new reference due out momentarily from CRC Press, will be of interest (Powell and Bagyaraj, 198_).

Besides these recent reviews, several points that were to be considered have surfaced in other segments of the meetings. Hence, we propose to minimize repetition/redundancy by truncating coverage herein to include a) a few summary items from example references--broadened slightly to emphasize some neglected or overlooked aspects of certain soils, b) brief mention of some "in progress/in press" and presumably "cutting edge" work from scientists willing to share items of interest with us now, and c) a vote to encourage support of some adventurous un-vogue mycorrhiza researchers. Our information sources and orientation relate mainly to endomycorrhizal systems, although similar principles and mechanisms may be involved in ectomycorrhizal associations as well.

The dominant critical roles of P in the energetics of host plant and VAM symbiont alike greatly complicate attempts to assess impact of VAM on uptake of, and subsequent growth responses to elements other than P. Efforts to first separate out the effects due to P itself by manipulating fertilizer P only in non-VAM controls to get "matched" plants (i.e., size, physiological age/maturity and internal P concentration equal to a non-P-fertilized VAM plant) suffer difficulties in data interpretations because of unknown effects from the dissimilar treatments. Be that as it may, when the plant tissue has been analyzed for other elemnts, reports often show that the mycorrhizal plants contain significantly different--sometimes higher but sometimes lower--concentrations, total accumulations, or both, of several elements than do the non-mycorrhizal control plants (Menge et al., 1982, Table 1; Yost and Fox, 1982). Unusually high concentrations, but small total accumulations of various elements often appear in slowly-growing non-VAM control plants stunted by environmental stresses such as excesses or deficiencies of nutrients, salts, water, temperature, light, and diseases. Conversely, lowered concentrations but large (relatively) total amounts of various elements are common in "growth enhanced" VAM-colonized plants. The biomass "dilution effect" as a contributing cause distinct from direct effect of imposed treatment variables must be considered first as a possible explanation for such shifts or apparent differences (Jarrell and Beverley, 1981).

a. Illustrative summary items

The same well-known physical/chemical/biotic factors controlling solubility, mobility, availability, and hyphal uptake of P should apply in similar fashion to the function and effectiveness of mycorrhizas for other "strongly soil-sorbed, immobile" elements. Indeed, firm evidence is available for at least 2 of these, leading Tinker and Gildon (1983) to conclude that "...it is quite certain that Cu and Zn supplies to plants can be markedly improved by mycorrhiza formation, and it may well be found that this applies to other elements also". Unfortunately, except where such labeled atoms can be traced unequivocally, it is seldom clear whether sorption of the "other" element was directly via fungal hyphae or indirectly via altered root membranes/structures. [Whether the mycorrhizae-aided uptake is beneficial, commensal, or deleterious to the host is a separate story not treatable in our brief time/space allotment.]

In parallel fashion to labeled Cu and Zn, evidence shows, as expected, that sulfur is another element sorbed into and translocated through VAM fungal hyphae (Gray and Gerdemann, 1973). One interpretation posits that this is of doubtful value to the host because highly mobile sulfur anions can reach the root itself at an adequate rate. Careless extrapolation or uncritical acceptance of such comments overlooks important properties of different soils. While ready mobility of sulfate and nitrate may be true for soils whose active-fraction clay mineral and humic colloids have a preponderance of net negative charges, please recall that there are substantial acreages of soils with x-ray amorphous hydroxy iron and aluminum components and allophanic or pseudo-crystalline imogolite-type clay

materials. Such soil colloids may have enormous surface areas, appreciable net-positive charge or anion exchange capacities, and behavior unlike more common crystalline-clays that dominate many agricultural soils. Mobility of sulfate and nitrate in these soils (that are derived from, or heavily influenced by, volcanic-ash parent materials) can be markedly reduced. Should we not expect mycorrhiza to be significantly effective under those conditions?

To emphasize with more specific examples, consider a class of soils called Andisols. Although little known to most agriculturalists, they are of considerable importance to people who obtain sustenance from them. Andisols are quite common in Pacific rim, as well as other, countries--including west coast areas of South and North America, up around to Japan, and on down to New Zealand. Common characteristics of the soils include large cation exchange capacities, very strong acidity, very low nutrient-base saturation, extraordinary accumulations of organic carbon, high iron content, and large P-fixing capacity. Despite the high O.M. content, acidity, and Al, they are not devoid of mycorrhizas despite possible expectation as reasoned from the mycorrhizal literature. The point is, that in our zeal to simplify and generalize--whether for convenience, for hurried publication, or for categorizing and managing information to better cope with the awesome diversities and similarities in nature--let us not push too far, too fast, with unproved or limited-area-application generalities.

b. Example excerpts from current/recent work, and mention of some knowledge gaps.

Participants may wish to contact authors or watch for formal publication of these in-pipe-line results on "other elements".

Intriguing effects of boron and magnesium on both ecto- and endomycorrhizas have been noted at the University of Missouri (R. J. Mitchell et al., 6th NACOM Poster summaries, this volume). Some of their studies involved foliar applications of various combinations of Mg, B, Mo, Fe and Zn in conjunction with basal NPKS macronutrients. Under conditions of low soil P, foliar application of zinc and iron chelates reportedly increased nutrient concentrations of rough lemon foliage without significantly increasing seedling growth or VA mycorrhizal development. By contrast, foliar application of Mg (100-300 ppm) and boron (50 ppm) increased seedling VAM development, foliage nutrient content, and growth. Other studies involved walnut, soybeans, oak, and short leaf pine. Foliar applied B was considered excellent for stimulating mycorrhizae, and was somewhat better for ectos than endos; Mg seemed better for stimulating endomycorrhizas. Possible modes of action were through effects of increasing IAA content and element transport across membranes, among other things.

Rather extensive greenhouse experiments have considered the influence of Al and the heavy metals Fe, Mn, Zn, Cu, Pb, and Cd on development and efficacy of vesicular-arbuscular mycorrhiza in several tropical and subtropical plants, with part of the studies involving several soil pH levels and soil water regimes in different combination (B. Fabig, 1982).

VAM inoculation allowed better growth of all test plants irrespective of Al, Fe, Mn, Zn, Cu, Pb, and Cd up to a soil application level specific for each element and beyond which mycorrhizal efficacy was more or less inhibited. The tolerated concentration levels were quite large such that even the highest amounts of Al, Fe, Mn, Zn, and Cu did not impede VAM infections nor cause microscopically visible injuries; only toxic levels of Pb suppressed VAM infections, by ca. 50%. Evident morphological changes were: increased development of vesicles associated with high amount of Pb; and extensive development of arbuscules--to the extent of being crowded/close-packed--associated with high amounts of Cd. VAM inoculated plants sorbed more P, Al, Mn, Zn, Cu, and Cd than did controls; and accumulations or concentrations were greater in the roots than in shoots except in single cases. (For instance, Pb concentrations in VAM roots were significantly lower than in uninoculated plants--biomass dilution effect?). Larger amounts particularly of Mn, Zn, and Cu were sorbed at pH 4.5 by inoculated Capsicum plants from nutrient deficient soil media. However, toxic levels of Fe in the soil did not give higher Fe concentrations in either roots or shoots of VAM plants.

Reports that VAM can either enhance or inhibit uptake of Mn further illustrate need for more work and clarification. For example, VAM seemed to decrease Mn uptake---and thus protect soybeans against toxic levels in acid soils--whereas Zn and Cu concentrations were increased in both roots and leaves of the colonized soybeans (Pacovsky et al., 6th NACOM Poster summary, this volume). In separate short (4-6 wk) greenhouse studies, VAM-seedling geraniums had only 1/3 as much foliar Mn as did non-VAM controls, and apparently not due to growth dilution effect (Biermann and Linderman, 1983).

Contrastingly, Huang and Yost (personal communication, 1984) are seeing a different picture for several legumes grown on an acidic, high-Mn soil (Wahiawa series, Propeptic Eutrustox) from Poamoho Research Farm, Univ. of Hawaii. Legumes included Leucaena leucocephala, Pueraria phaseoloides, Desmodium ovalifolium, Desmodium infortum, Glycine max, and Vigna unguiculata. On unlimed soil (pH 4.9-5.4; water soluble Mn = 10-15 mg/L), top growth was depressed both in mycorrhizal and non-mycorrhizal plants. Depressed growth was attributed to Mn toxicity as evidenced by visual symptoms and exceedingly high Mn concentrations in foliar tissue (1000 to 5000 mg Mn/kg depending on plant species). Where lime was applied, plant biomass and shoot P concentrations of mycorrhizal plants were much higher than for non-mycorrhizal controls. However, with certain VAM species, accumulations of Mn were greater in, and toxicity symptoms were more intense on, the mycorrhizal plants. Whether VAM fungi directly enhance the Mn sorption and accumulation is currently under study.

Remarkably high concentrations of Mn in non-VAM and VAM colonized sweetgum seedlings (2000 to 6000 ppm) grown on a strongly acid Jory soil (pH 5.0;

Xeric Haplohumult) were observed earlier by Davis (1982). One might expect such concentrations to b toxic to both sweetgum and VAM symbiont, and apparently became so for the plants one weekend when they came under severe evapotranspirational stress from a sudden heat-wave. Curiously, however, when P starvation of the seedlings grown in this very P-deficient soil was alleviated by Glomus fasciculatum, the healthy seedlings, and apparently the VAM symbiont, tolerated exceptionally high levels of Mn. Clearly, the VAM colonization (Gl. fasciculatum, or Gl. mosseae) did not aid the roots to exclude Mn; whether the symbionts aided Mn uptake cannot be told from the data, but were not obligatory since non-VAM seedlings also showed high leaf Mn concentrations. Other literature suggests that sweetgum may be something of a Mn accumulator, given suitable circumstances.

Further contrasts in the Mn story are seen in observations that seedlings of Solanum opacum grown on sandy Australian soil amended with Mn^{+2} (75 to 150 ppm) showed significant correlation between shoot tissue Mn concentration and % VAM colonization (P. McGee, 6th NACOM Poster summary, this volume). Clearly not all plant, VAM fungi, and soil systems behave the same.

Further additions to the nitrogen-VAM stories are on the horizon and need reconciliation. For example, effects of VAM systems on nitrogen movement or nitrogen enzymes in onions at various P-treatment levels appear minimal. Observations so far in this Australian work are interpreted as showing no evidence that the fungi take up N directly; effects still are believed related to P nutrition of the host (S. Smith, 6th NACOM, verbal comments). By contrast, studies with nitrogen-15 labeled ammonium sulfate (and sorghum tissue) placed 5.5 cm from celery (Apium graveolens L.) roots showed ^{15}N transport through hyphae to roots and some translocation to celery tops within 3 to 6 days. An average of 25% of the applied ^{15}N appeared in the plant by 30 days, despite just 6% colonization of roots by Gl. mosseae. There were no significant differences in top dry wts. or in P concentrations or contents between VAM and control plants (Ames et al., 1983).

The ability of certain VAM strains/species to tolerate ordinarily toxic levels of Zn contrasts with great sensitivity of other strains to Zn and some heavy metals (e.g., Cd) and needs further elaboration. Our hopes of learning more about reasons for differences between a Zn-tolerant Gl. mosseae strain from a coal-spoil area and a Zn-sensitive Gl. mosseae strain from Rothamsted fields faded quickly on learning from Dr. Tinker that the work was phased out on completion of A. Gildon's thesis studies. Disappointingly, little additional information is being developed beyond what has been published (Gildon and Tinker, 1983).

Similarly, more insight is needed as to how some VAM fungi tolerate seemingly toxic levels of exchangeable Al in strongly acid soils (such as in Andisols) while others may not. Whether greenhouse stock G. caledonicum (cultured on leeks at pH 7.5) which was strongly inhibited by sudden exposure to Al in sand-solution culture at pH 4.5, would be equally inhibited by the same soil-solution Al concentrations in soils in the field remains to be seen (Wang et al., 6th NACOM Poster summary, this volume). Is the same inhibition of G. caledonicum (G. caledonium?) expressed when other bioassay host plants are used? Adapted or naturally acid-tolerant mycorrhizal fungi seem likely to have quite different Al tolerance levels, even if the patterns of inhibition/toxicity turn out to be similar.

What are the means by which some mycorrhizal systems tolerate extraordinarily unbalanced ionic conditions such as in salty soils, or in serpentine soils with their very high Mg and low Ca concentrations. Mycorrhizas develop on paper birch in the face of elevated levels of copper and nickel (Jones and Hutchinson, 6th NACOM Poster summary, this volume). Other plants/shrubs thrive on high nickel-content soils (northern California and southern Oregon) and accumulate astounding levels of Ni in their tissue (some > 10,000 ppm). In fact, numbers of plants bioaccumulate or tolerate exceptional levels of various metals and thereby serve as indicators in biogeochemical prospecting. The presence, function, durability, or effectiveness of mycorrhizas in such systems is largely unknown. Where mycorrhizal components do exist, perhaps much could be learned about how mycorrhizal roots deal with these severely imbalanced, stress conditions. Isolates obtained from such severe-stress sites might be effective in reclamation of scarred, waste-spoil, or otherwise polluted lands.

Effectiveness of mycorrhizal systems, whether by enhancing or inhibiting uptake of elements, may become better understood through studies with more exotic heavy metals and other toxicants from society's wastes, and reckless ways with natural resources. For example, VAM fungi apparently effect differential uptake of ^{137}Cs and ^{60}Co depending on whether the host is a representative legume or grass. The studies inovlved three inoculum sources and soil contaminated 7 years previously at AEC sites near Arco, Idaho (R. Rogers and S. Williams, personal communication and oral presentation, 6th NACOM, 1984). After 2 to 4 months, Cs content in clover with VAM was double that of the control; but in Sudan grass, Cs content was not significantly different between VAM treated and control plants. The uptake was reversed with Co; i.e., in clover, Co content in the VAM and controls was not significantly different, but in the mycorrhizal grass, Co was 2-1/2 times greater than in controls.

Knowledge gaps remain with respect to whether deficiencies or toxicities result directly from VAM activity or indirectly via other elements, principally P. What other growth-inhibiting imbalances can be induced by nutrient additions that suppress VAM, such as so-called P-induced Cu and Zn deficiencies? In what forms are the metals and micronutrients sorbed and what are the mechanisms by which toxic elements are excluded/inhibited by VAM roots systems (Babich and Stotzky, 1983b)? Note that Cu toxicities in relation to mycorrhizae on citrus has shown up in Florida studies (J. Graham, personal communication, 1984).

Effectivity vs. infectivity of VAM species needs much more documentation. Demonstrably, certain VAM species/strains, hosts and combinations are superior under given conditions, and in that sense there is VAM-host specificity. Considering the large variability in vigor, strength, capacity, environmental tolerance, disease resistance (even 'talent', if you will), of individuals within and among virtually all species of organisms, why shouldn't we expect there to be superior VAM isolates/strains, also? Crucial to a better understanding of effectiveness or superiority of any VAM fungal isolate are answers to critical questions about hyphal characteristics, proliferation pattern, soil exploration tendency and capacity, zone(s), duration, and efficiency of active nutrient uptake, and relative energy cost to the host. How can hyphal ramification into field soils of greatly different tilth and structure be observed and determined in situ? What level of infection is optimum and what is excessive--the greater the % infection, the greater the carbon drain from the plant? How much infection is needed when, and for how long? How does this vary for different VAM species/strains and hosts? Early calculations (Bielski, 1973) indicated that relatively few entry points and low % colonization levels could be adequate for supplying sufficient P to the host.

Much more needs to be learned about effectiveness of mycorrhizae for plants beyond their being scavengers and pumps for P and similarly immobile micronutrients. In that connection, what is the effectiveness or function of VAM fungal isolates that colonize plants despite high levels of available P, and what are the consequences of sometimes rather extensive external hyphal development concurrent with low % colonization of roots (Davis et al., 1984; Young et al., 1985).

One approach to estimating the relative dependence of a plant on mycorrhizal fungi, hence potential effectiveness of the VAM symbiont, has been suggested by the coauthor (W. J.). The approach involves ranking the elements in the following manner: 1. Estimate the average minimum whole-plant concentration required for normal development of the plant, expressed as C_{avg}, in moles/kg. 2. Measure the soil solution concentration of the element, expressed as S_{avg}, in moles/liter. 3. Divide C_{avg} by S_{avg}, and rank ratios accordingly. This ratio provides a measure of the relationship between plant demand and the supplying power of the soil through mass flow alone. Although by itself it cannot indicate directly whether or not an element will be limiting for the plant, it can provide an index of relative limiting supply from soil to the plant. While this technique takes into account the effects of transpiration ratio, it also assumes that the concentration in the soil's solution changes relatively little throughout the growing season. Thus, it would be less suitable for N, Fe, or Mn than it would be for P, Zn, Cu, Mo, and other nutrients which are relatively well-buffered in the soil solution. The technique also requires that the relative affinity of the fungus for the various elements is the same, or, if different, can be factored into the relationship. In most instances where plants respond to mycorrhizal fungi, this technique suggests that P should be the most limiting element, followed by either Zn or Cu.

Under conditions where stresses other than nutrient availability are also imposed on the plant, increases in concentration due to mycorrhizal fungi may become greater at higher levels of stress. For example, Ojala et al. (1983) found that under low salinity, VAM had 1 ttle effect on plant K concentrations; but at high salinity, mycorrhizal plants contained greater concentrations of K than did non-mycorrhizal plants. This effect may have been due to the enhanced P nutrition directly, or to some other effect of the mycorrhizae.

With all the foregoing, it bears reemphasis that results from laboratory studies using artificial media can be seriously misleading in relation to what happens in soils in the field (Bowen, 1984). The same caution must be emphasized for results from many, perhaps most, greenhouse experiments. Extrapolations from short-term greenhouse trials using small-volume containers and limited-range environmental parameters are especially hazardous in attempts to predict effectiveness of mycorrhizal systems in real-world field situations.

c. Vote in support of some un-vogue adventurous mycorrhiza researchers.

Mycorrhiza research seems not to be immune to the trendy-topics, herd-instinct pressures that bedevil other research fields. Unfashionable, unconventional observations and efforts tend to get ignored, buried or lost. Rather than dismiss the unusual, the small, the presently uneconomic or seemingly isolated cases, surely we should encourage at least some mycorrhizae enthusiasts to follow the sage advice from knowledgeable plant breeders, i.e, "Learn to treasure your exceptions!". Therein may lie important answers.

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THE PROBLEMS OF LIVING WITH A PLANT ROOT: FACTORS INVOLVED IN ROOT COLONIZATION

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Keywords--rhizosphere, rhizoplane, elicitor, suppressor, preformed defense barriers

Contact between plant roots and a wide array of microorganisms is inevitable as growth proceeds through the soil. Colonization of the root may occur and produce a range of effects. Most dramatic and costly are the deleterious interactions with pathogens. The structure and functioning of the root may be sufficiently altered to cause total plant necrosis. Beneficial interactions also occur. The effects are often visable because of an improved growth form. With some beneficial organisms, such as Frankia, Rhizobia and the fungi that form mycorrhizal structures, intricate growth of the organism within the root cortex is required. This symbiotic growth pattern raises questions as to how the interactions are established and maintained. A common adage of plant pathologists is that plants possess highly efficient mechanisms of defense to limit microbial invasion. Yet these mechanisms clearly are as ineffective for preventing the growth of symbiotic microorganisms as they are for the root pathogens. This presentation discusses areas of research that seem fruitful for understanding the specificity of plant-microbe interaction. The zones and the molecular language of communication between the plant root and the microorganism will be addressed.

In the interaction between the root and the mycorrhizal fungus, three zones of intercommunication will be considered. These zones are the rhizosphere, the rhizoplane, and the root cortical cells. At each zone there are molecular exchanges which could be determinants in whether or not the fungus proceeds towards producing a mycorrhizal structure.

The Rhizosphere

The key event in the rhizosphere is the survival of the mycorrhizal fungus. Survival involves successful competition with other soil microorganisms and negation of potential resistance factors eminating from the plant root. Viability of the fungus is dependent on adequate water and nutrient supplies. The fungus most possess the enzymic machinery to utilize the nutrients available from organic matter and the root exudates. In competing with other microflora, the production of siderophores, to secure iron from the environment, may be significant (22). The secretion of antibiotics to limit growth of other organisms may be involved (14, 15). The orientation of the fungus towards symbiosis within the plant root may be speerheaded by the production of plant hormones. The synthesis of cytokinins, ethylene, and indole acetic acid (IAA) has been documented for certain ectomycorrhizal fungi (11). These hormones may alter the morphology and functioning of the root. Bifurication of the root may be enhanced. Root exudation, and hence nutrient and water supply to the fungus, may be promoted through altered permeability of the membranes of the root cells.

In addition to competition with other soil microbes, the mycorrhizal fungus must resist potential defense strategies of the root. One defense system that extends into the rhizosphere may be the exudation of toxic components, frequently as protected glycosylated structures (19). Glycosidases produced by the rhizosphere microbes or present in the root exudates could release the toxic aglycone in the rhizosphere. If mycorrhizae exist on plants that produce such toxins, the fungi probably have evolved systems of immunity to the factors. The hyphae may lack systems for uptake of the components or they may have sufficient detoxification mechanisms.

The Rhizoplane

Survival at the rhizoplane involves the same problems that have been addressed for the existence of the fungus in the rhizosphere. What is the nature of the nutrients utilized by the fungus? Is the production of siderophores and antibiotics significant in the competition between the mycorrhizal fungus and other colonizers? How are the defense systems of the plant root avoided?

Recent work in our laboratory has suggested that a defense barrier based on peroxidase activity on the root surface may require circumvention. The root surface of several plant species examined demonstrate potent peroxidase activity. In the presence of hydrogen peroxide, the peroxidase would oxidize phenolics to produce phenoxy radicals that have antimicrobial activity. These radicals too may condense to produce lignin polymers that can encrust the plant cell wall and impair the action of potential degradative hydrolases (9, 23). Hammerschmidt and Kuć (9, 10) have suggested that lignin may also be deposited on mycelial walls. Such deposition would limit further growth of the fungal hyphae. A second potential defense role of the peroxidase is the production of superoxide anion, O_2^-, and hydrogen peroxide. This resistance mechanism would be analagous to the function of peroxidase in leukocyte cells in the mammalian immunological defense system (3). In the root system, generation of superoxide anion and hydrogen peroxide accompanies the peroxidase-catalysed oxidation of NADPH and NADH. The NAD(P)H oxidation requires a divalent metal ion and a monophenol as cofactors (8). In our assays Mn^{2+} and p coumarate are satisfactory. Malate dehydrogenase has been detected in root wall preparations and consequently could provide the natural source of NAD(P)H (8). Superoxide and hydrogen peroxide are potent antimicrobial compounds for organisms that lack enzymic systems for their destruction. Consequently, if the peroxidase activities constitute an effective defense system, the mycorrhizal fungus should possess catalase and superoxide dismutase activities. A third potential role of the root surface peroxidase is that certain of the isozymes demonstrate IAA oxidase activity in the presence of Mn^{2+} and a monophenol. This activity may aid in negating the effect on the plant root of IAA generated by soil microorganisms such as mycorrhizal fungi (11).

Since peroxidase is an iron containing enzyme, it is tempting to speculate that the siderophores

produced by the mycorrhizal fungus (22) may not only be functional in competition with soil microorganisms but also with the plant host. Chelation of the iron from the peroxidase by a siderophore would impair the putative defense response that are based on this enzymic activity.

The successful symbiosis of the mycorrhizal fungus may require additional molecular ploys to establish colonization of the root surface. Recognition between surface components on the challenge and on the plant root may be significant. Attachment of Agrobacterium cells to wound areas may involve interaction between the bacterial lipopolysaccharide (LPS) and pectin (13). Binding of Rhizobium may require plant lectin recognition of bacterial surface polysaccharides (4). Attachment of Phytophthora zoospores (16) or pseudomonad species (2) to the plant surface may involve interaction with plant agglutinins that are hydroxyproline-rich glycoproteins. Similar recognition processes may be occurring with mycorrhizal colonization. Piché et al. have reported that colonization of a host enhances the amount of polysaccharide material that encrusts the hyphae. These workers propose that the polysaccharides could act as "a cement" to anchor the fungus to the plant surface.

These possible enzymatic and recognition processes suggest that the rhizoplane is far from just an inert surface that can be readily colonized by any microbe. Rather we suggest that there are several stages of molecular coevolution which specifically promote colonization by the mycorrhizal fungus.

Cellular Responses

The mycorrhizal fungus eventually produces hyphae that ramify into the cortex. Penetration demands that the epidermal suberin barrier be breeched. Relatively few studies on penetration events are reported, but entry at wound sites, natural openings, and by appressorium formation are documented (20). Studies with ectomycorrhizal fungi demonstrate that in tree roots the hyphae generally bypass the epidermal cells containing tannin accumulations (18). Avoidance of this preformed chemical barrier is essential for the further growth of the hyphae into the cortex. The interaction between the hyphae and the epidermal cell walls is regulated so that penetration does not occur, and the tannins are not released through extensive wall disruption.

The relative contribution of mechanical pressure and enzymic dissolution to the process of hyphal growth through the plant cortical walls is unresolved. Involvement of enzymic processes asks questions of the nature and regulation of the enzymes involved. Is a pectinase the significant enzyme? How are activities regulated so that complete dissolution of wall structure avoided? Do hormones produced by the fungus alter the integrity of the wall structure and promote wall softening?

The intimate growth of the hyphae with the cortical cell raises the problem of how the fungus fails to elicit an induced resistance response. Frequently a challenged plant cell undergoes dramatic metabolic changes, termed a hypersensitive response, which terminate further spread and microbial development. Different aspects of this pleiotrophic hypersensitive response may be important in restricting growth of different challenges. The response includes increased permeability of the plasmamembrane and tonoplast. Consequently, the reacting tissue eventually may demonstrate dessication. Phenolic metabolism is enhanced, partly by de novo synthesis of key enzymes. Lignin may be deposited around the walls of the responding plant cell and prevent penetration of the organism into surrounding healthy cells. The accumulation of oxidised polyphenols and low molecular weight phytoalexins may impair the functioning of the invading organism.

Study of plant-pathogen systems has demonstrated that these changes characteristic of a hypersensitive response may be simulated by treatment of plant tissues with microbial components termed elicitors. Several categories of components have elicitor activity, including hyphal wall glucans, chitin and lipids (1, 5). Certain enzymes which degrade the pectic component of the plant cell wall release pectic fragments which themselves are elicitors (6). We have demonstrated that two species of ectomycorrhizal fungi, Rhizopogon vinicolor and R. occidentalis produce elicitors. Elicitor activity is detected in culture filtrates and in saline washes of mycelia. The activity is demonstrated on bean cotyledons, a non-host tissue, as well as on callus initiated from Douglas-fir roots. Douglas-fir roots are incompatible with R. occidentalis but compatible with R. vinicolor.

Control by the challenge over the initiation of the hypersensitive response would ensue if the elicitor was detected only in noncompatible tissues. The elicitor alone could display host specificity or the microbe may produce an additional factor, a suppressor, to mask elicitor recognition in compatible tissues (7). Both of these schemes are applicable to the interaction of plants with vesicular-arbuscular (VA) or ectomycorrhizal fungi. With VA mycorrhizal fungi compatibility is the general response. Perhaps these fungi do not produce elicitor active components. Alternatively the VA fungi may produce an abundance of suppressors. Certain ectomycorrhizal fungi are reported to display distinct specificity patterns (17). The symbiotic pattern may be explained by the production of specific elicitors or the synthesis of suppressors which regulate the detection of nonspecific elicitors.

Another consideration for the mycorrhizal relationships is that some of the reactions characteristic of the hypersensitive response may occur yet without causing necrosis of the symbiotic fungi. Clearly the compatible plant cells contacted by the mycorrhizal fungus do not demonstrate the rapid necrosis typical of the hypersensitive response. However, accumulation of phenolic components in compatible mycorrhizal tissue is reported. Cortical cells of ectomycorrhizal Douglas-fir roots display accumulations of tannin-like components (21). Orchinol and other metabolites with phytoalexin activity are synthesized in mycorrhizal orchid roots (11). The mycorrhizal fungi may possess some tolerance to the induced chemicals or contact between hyphae and the chemicals could be limited. Alternatively, the

chemicals may provide an ecological advantage for the adapted mycorrhizal fungi over other sensitive root colonizing microorganisms. These observations on induced changes in phenolic metabolism suggest that elicitor-like compounds may be functional even in the successful symbiotic relationship.

The concepts presented in this discussion illustrate that the formation of the mycorrhizal structure is not an easy accomplishment. The plant has evolved effective methods to prevent general microbial colonization of the root. The mycorrhizal fungus must survive through the maze of the rhizosphere, rhizoplane, and plant cortical tissue without falling through a trapdoor into a inescapable resistance system. It is our exciting challange to prove the molecular language which permits the mycorrhizal fungus to establish a symbiotic relationship with the plant root.

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EARLY EVENTS IN ROOT COLONIZATION BY MYCORRHIZAL FUNGI

By

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Keywords--Ectomycorrhizae, vesicular-arbuscular mycorrhizae, Glomus monosporum, Pisolithus tinctorius, growth pouches, seedling transplant.

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Introduction

In order to study the early events in the colonization of roots by mycorrhizal fungi, reliable and efficient methods for mycorrhizal synthesis are needed. Plants must be grown in such a way that mycorrhizal roots can be sampled for microscopy or physiological studies with minimal disturbance to the association and with little interference from growth medium. This presentation describes techniques that have been developed to study all stages in root colonization for vesicular-arbuscular mycorrhizae (VAM) and ectomycorrhizae. Examples of the types of information that can be obtained using these techniques are included.

VAM

A transplant technique (Brundrett et al., 1984a) in which leek (Allium porrum L.) seedlings are transferred to well-established dual cultures of Glomus monosporum Gerd. and Trappe - leek growing in turface, leads to rapid colonization of roots (Table 1). Hyphal adherence and appressorium formation first occurs within 1-2 days after seedling transplant and at an average of 6 mm behind the growing apex. Appressoria usually develop along adjacent epidermal cell walls rather than over the outer surface of an epidermal cell. Penetration of the hyphae into the root also occurs within 2-4 days (Table 1) and at a mean distance of 11 mm basipetal to the root apex. Depending on the anatomy and age of the root, intracellular hyphae may be confined to particular cells in the exodermis, the outer layer of root cortical cells. Many monocotyledonous species, including leek and asparagus, have roots with a specialized exodermis, the cell walls of which become suberized. In asparagus, intracellular coils are restricted to short cells which develop suberized cell walls later than adjacent long cells in this layer (Hussey, 1982). In leek, asparagus and other roots, cell walls of the epidermis may become modified as well. Wall modification of outer layers of roots, therefore, must be taken into consideration when studying root penetration by VAM fungi.

Subsequent to penetration of the root by hyphae, root structure can still influence VAM development. For example, in species like leek, well-developed intercellular air channels provide free space for rapid growth of intercellular hyphae, obvious by 2-4 days after seedling transplant (Table 1).

Arbuscules are initiated at a mean distance of 18 mm from the root apex with a lag of 1-2 days after root penetration (Table 1). Many lateral projections form along intercellular hyphae, only some of which penetrate cortical cells and initiate arbuscules. Cleared roots stained with chlorazol black E and observed with Nomarski interference contrast microscopy (Brundrett et al., 1984b), provide details of arbuscule development, at the light microscope level, not reported previously.

Functional arbuscules occur as early as 5 days after transplanting leek seedlings into the dual culture pots (Table 1). Senescence of arbuscules was observed at approximately 8 days (Toth and Miller, 1984). Vesicles also form within 5 days and along infection units that are still initiating arbuscules.

The seedling transplant system provides abundant mycorrhizal roots at all stages in the colonization process so that now it should be possible to design experiments to determine the mechanism of adherence of VAM fungi to root surfaces. Also, the ultrastructural events which occur during appressorium formation, hyphae penetration, arbuscule ontogeny and vesicle development, can be determined using VAM synthesized by this procedure.

Ectomycorrhizae

The growth pouch method (Fortin et al., 1980) has facilitated the study of early events in the colonization of white pine roots by Pisolithus tinctorius (Table 1). Contact of hyphae with short roots and subsequent colonization of the surface of these structures occurs within 1-2 days after placing P. tinctorius inoculum in the pouch (Table 1). Within 2-4 days, rapid development of interwoven hyphae results in the formation of a mantle around short roots, leaving the long root surface nearly free of hyphae. Intercellular penetration of hyphae and the formation of a Hartig net also occurs during this time period (Table 1). These events are correlated with changes in the short root apical meristem induced by the colonization of the root. Central cells of the original meristem vacuolate and two lateral meristems form which subsequently lead to root dichotomy (Piché et al., 1982).

Piché et al., (1983a) provide details of ultrastructural changes during ectomycorrhiza formation. During early Hartig net formation (2-4 days) there are already changes in intercellular contact in the cortex since hyphae grow into the middle lamella. With continued development of the Hartig net, cortical cell shape and cytology are altered. Cortical cell cytoplasm undergoes necrosis and symplastic continuity may be interrupted since plasmodesmata are not observed at this stage. The latter point needs clarification since the relationship between cortical cells determines whether carbohydrates produced by the host move to the Hartig net - cortex interface in the apoplast or symplast.

Table 1. Methods of rapid synthesis of VAM and ectomycorrhizae in controlled environments

Days	VAM (Glomus monosporum - leek) Transplant procedure	Ectomycorrhizae (P. tinctorius - white pine) Growth pouch technique
1-2	adherence of hyphae on root surface; appressoria	attachment of hyphae on root surface
2-4	penetration of hyphae into root; presence of inter- and intracellular hyphae; arbuscule initiation (3-4 days)	interwoven hyphae on short roots; mantle and Hartig net ontogeny
5+	arbuscule maturation and senescence; vesicle development	dichotomy of short roots

Compatibility between root and mycosymbiont

Treatment of this topic will be confined to ectomycorrhizae. Secretory substances produced by both the root and the fungus may be important in the early attachment of hyphae to the root surface (Piché et al., 1983a,b). Ectomycorrhizae synthesized between P. tinctorius and white pine in growth pouches provide good material for observations of the earliest stages of adherence of hyphae to short root-surfaces. At this time (1-2 days after inoculation) polysaccharides are evident after treating fixed and oxidized roots with silver proteinate (Thiery reaction). This technique, combined with backscatter electron imagery and energy dispersive spectrometry using the SEM (Piché et al., 1983b) shows that polysaccharides are present on short root and hyphae surfaces. Sections of similar material show that only those fungal hyphae close to the root surface produce polysaccharides that react with the Thiery reaction (Piché et al., 1983a). Walls of these hyphae are altered and lomasomes, which are Thiery-positive, appear in the cytoplasm of these hyphae (Piché et al., 1983a).

Since a carbohydrate-carbohydrate adhesion mechanism may be involved in the establishment of ectomycorrhizae, more advanced cytochemical approaches are being used to investigate this possibility. RNase-gold is being used to assess, for the first time, the usefulness of colloidal gold markers on resin-embedded plant roots (Piché et al., 1984). In addition, morphometric and X-ray analysis techniques are used to localize and quantify the RNase-gold bound in root meristem cells (Piché et al., 1984). With this background information, other proteins such as lectins and antibodies are being labelled with colloidal gold to characterize the secretory sugars present on root and hyphal surfaces. As a preliminary to studying pine, these techniques are being applied to characterize the secretory products of non-mycorrhizal Zea mays roots since the carbohydrate chemistry of this system is well known (Rougier, 1981).

It is obvious that new methods such as colloidal gold marker techniques, coupled with the growth pouch system, should be used to unravel the complexity of adherence of ectomycorrhizal fungi to roots.

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PHYSIOLOGICAL FACTORS IN INFECTION AND SPREAD OF VAM

by

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Keywords Specificity, recognition, exudates, fungal growth substrates

Introduction

Other papers in this conference have examined environmental factors affecting the development of vesicular-arbuscular mycorrhizas (VAM), and modelling the development of the symbiosis. In this paper I address some of the physiological or mechanism aspects of these. We may be interested in mechanisms for purely scientific curiosity reasons or for managing the symbiosis more effectively. One might argue that as there is little specificity in the infection (differences in infection rate appear to be related principally to time for germination of propagules), understanding the infection process will not enable us to help the plants to "do it better". This may be much too narrow a view for there are a number of economically important plants which do not form VAM and furthermore understanding why most plant species do not reject the infection may be important in managing other plant microorganism associations. Understanding what controls the spread of the infection, its longevity in any part of the root, and what "drives" fungal growth in the root and in soil could have very great benefit to managing the symbiosis.

I have structured this paper with regard to two major phases of the symbiosis - infection processes (and recognition) and the growth and longevity of the association in the root. Much research has confused these two distinct factors. In a number of cases conclusions on infection processes are drawn from data on the percent of root infected (which has components of both phases) rather than numbers of entry points. Drawing a distinction between susceptibility of particular parts of the root and the substrates available to the fungus for growth is basic to understanding such phenomena as the apparently greater ease of infection of younger parts of the root which has been indicated by the Smith-Walker model (1981) (but not yet experimentally tested), and to the understanding of observed differences in the root cortex with respect to arbuscule development (see Gianinazzi-Pearson, 1983). Are possible cell wall differences in different tissues responsible for differences in VAM development within a root or does the abundance of growth substrates (perhaps including oxygen levels) differ?

Infection and "Recognition"

Most research to date has been on the details of cortical cell infection and subsequent arbuscule development; excellent electron-microscope and allied histochemistry studies have been made of this (see Carling and Brown, 1982; Scannerini and Bonfante-Fasolo 1983; Gianinazzi et al. 1983), and I shall not elaborate on these (however see Bowen, 1984). Gianinazzi-Pearson (1983) has presented a very thoughtful essay on host-fungus specificity, recognition and compatibility in various mycorrhizal types.

Concepts of recognition. What do we mean by 'recognition'? It is becoming a very fashionable word. To some it refers to specific situations such as the narrow host specificity developed between rhizobia and legumes, or perhaps the rather more coarse specificity between a number of basidiomycetes and ascomycetes and the relatively small group of plants forming ectomycorrhizas. That is, there are host specificity, recognition or receptiveness factors involved. This connotation of recognition may have rather less meaning for the relatively non-specific infections between VA endophytes and the enormous range of plants forming VAM. Another definition of recognition is that of Heslop-Harrison (1978), that "a cell that reacts in a special way in consequence of association with another must do so because it 'acquires' information from that other, information that must be conveyed through chemical and/or physical signals". Call it what you will, 'recognition' or 'plant reaction to infection', in the final analysis we are concerned with questions such as 'Why is there such lack of specificity in VAM?' and 'Why is the development of arbuscules frequently restricted to inner cortical cells?' Keen (1982) listed the requirements of successful pathogens and concluded that in mutualism with higher plants micro-organisms have acquired all necessary properties of both specialized and non-specialized pathogens. He comments that in mutualism, plant and microorganism have evolved such that specific recognition does not occur to evoke active host defence. Below I have listed many most of the characteristics of 'successful' pathogens he perceived, with my assessment (or sometimes a guess) of where the VAM association stands in respect to these (in parenthesis):

1. Pectic enzymes produced for maceration of host tissue (probable, see Bowen 1984).
2. Toxins for killing host cells (almost certainly does not occur with VAM).
3. Substances destroying permeability barriers (VAM do not destroy permeability barriers but they may well modify permeability - see below).
4. Degradation of preformed toxins or phytoalexins (possible but it is more likely they do not elicit phytoalexins or are insensitive to them - see Bowen, 1984).
5. Production of surface molecules which prevent detection of underlying polymers which would otherwise elicit a hypersensitive reaction (highly likely with VAM but needs more investigation).
6. Production of substances which suppress expression of host hypersensitive reactions (possible, but these substances would need to operate over a very wide botanical spectrum and would need to act on a very basic hypersensitive reaction).

7. Spore adhesion to the host surface. (Possible but is it necessary? This has been little studied with VAM, however see Gianinazzi-Pearson, 1983, for example in other mycorrhizas).
8. Formation of haustoria and other specialized structures (occurs with VAM).

As hypothesized by Sequira (1980) for Agrobacterium tumefaciens the wide host range for VAM would be consistent with non-specific recognition phenomena involving polymers common to many plant species. This is not inconsistent with the early evolution of VAM symbioses in relation to land plants (Nicolson, 1975).

The entry of the root. Compared with the development of arbuscules in the cortex, the penetration of the root at the root/soil interface has received little study. It is now fairly well accepted that the root (or root exudate) has little influence on stimulating spore germination.

It is now considered that increases in root exudation associated with phosphorus deficiency are not related to the infection process (Schwab et al. 1983a). However are there properties of root exudates which affect the infection process? If penetration of the root by VA endophytes is associated with enzyme action (as seems likely, Bowen, 1984) are these constitutive or induced by root exudates or root contact phenomena?

There appear to be no enzyme studies on external hyphae to act as controls for settling this question. Are there other 'messages' between the fungus and the root? It has recently been indicated that a protein in root exudates of soybean can stimulate early nodule formation in rhizobia (Halverson and Stacey, 1984) - could similar phenomena occur with VAM infection? If specific growth substances needed by the VA endophytes (in addition to energy sources) are synthesized by the plant, does it do this in response to a signal from the fungus even at this rhizosphere stage? It is possible, even probable, that the fungus also increases the permeability of the host plasmolemma (see later).

The 'inability' of certain plant groups to form typical VAM or even be infected has received little physiological attention and has been discussed elsewhere (Bowen, 1984). Toxin and fungus nutrition theories for this suffer from observations that VA endophytes can grow in the rhizosphere of 'non-hosts'. It may be that a fungus enzyme production necessitating root entry is not elicited by the non-host, that the enzymes are inactivated or that they are inactive toward the 'non-host' cell walls. The findings of Allen (1983) of apparently normal (but seasonal) infection of Atriplex gardneri, and infection following plant treatment with simazine (Schwab et al., 1982) indicate interesting tools for further study of resistance to infection.

Intracellular infection. To say the essence of VAM development is non-recognition by the host is too extreme a position. A number of changes occur to both the endophyte and the plant cell in arbuscule formation, some of which are reminiscent of plant-pathogen interaction but on a reduced scale. The fungus cell wall changes from a layered stratified wall to a monostratified wall. It thins considerably, and polymerization of N-acetyl glucosamine units to form chitin is blocked (Bonfante-Fasolo and Grippioli, 1982). The plant cell reactions include mild 'pathogen' reactions of collar production by the cell wall at the point of entry, sometimes some polyphenol production and disorientation of cell wall synthesis. Production of paramural bodies in a metabolically active interfacial matrix also occurs as do marked increases in cell metabolism, possibly associated with enhancement of production of cytokinins and other growth regulators (see Dexheimer et al., 1979, Bonfante-Fasolo et al., 1981, Gianinazzi et al., 1983, and Bowen, 1984). A progressive slowing of the rate of spread of the infection also occurs (data of Walker and Smith, 1984). In most cases the physiological stimuli for these changes are not yet known.

Fungal Growth Phases

The growth phases of interest in the VA infection are: (i) growth to the root, (ii) growth of the infection unit within the root (development of vesicles, arbuscules and intercellular hyphae) and (iii) growth of the fungus in the rhizosphere (ectotrophic spread) and into soil, leading to nutrient uptake and spread to neighbouring roots. I regard the availability of substrates and other factors for growth of the fungus to be a central issue in the above considerations, perhaps the main consideration. However 'resistance' to spread in the root rather than lack of growth substrate may be involved also in differences between fungus-plant combinations in the rate of spread in the root (see Bevege and Bowen, 1975; Buwalda et al, 1982), and between main roots and laterals (Walker and Smith, 1984). It must be said also that there is a general absence of experimentally determined data for rate of spread, obtained by point inoculation of parts of a root and followed by successive harvests.

Because of our inability to grow VA endophytes using only a range of substrates commonly used by fungi, we must assume for the time being that (as yet) unknown growth factors are also required. We have, therefore, two considerations: (i) this unknown factor(s) and (ii) commonly used substrates supplying energy. Because most research on root composition and root exudates focusses on easily assayed, common constituents of the cytoplasm, it is almost certain that only the second factor (commonly used substrates) is examined, not both factors. Such possible growth substances as glycerols, sterols and lipids have also been largely ignored.

Focussing on commonly used fungus growth substrates, what do we know of their use by VA endophytes? Not very much, but the studies of

Hepper (1983) and Hepper and Jakobsen (1983) on the effects of substrate on limited independent growth of hyphae give us a method for examining this. It would be interesting to examine the effects of root exudate fractions using the same techniques, for we know that hyphae will grow in the rhizosphere independent of infection (Bevege and Bowen, 1975). It is interesting that Schwab et al. (1983,b) have found a large increase in glycine and lysine in root exudates of P deficient sudan grass and that Hepper and Jakobsen (1983) found these (and cystine) stimulated growth of hyphae of Glomus caledonius considerably compared with several other amino acids/amides.

What of growth of the fungus before infection? There is a preferential growth of hyphae from germinating spores (and doubtlessly from other propagules in soil) toward the root. Powell (1976) found the extent of this chemotropism differed with the fungus - for one fungus it occurred for 3-4 mm and for two other species it was 1.6 mm. The specific or non-specific nature of the stimulus (stimuli) is not known. Koske (1982) found attraction of hyphae from germinating spores of Gigaspora gigantea to roots of beans and of corn by volatiles. These would diffuse over much greater distances from the root than would solutes and would be especially important as 'direction finders' for small numbers of large spores in soil with sufficient energy resources in the spore to grow to the root, provided there was some direction "message".

On arrival at the root the fungus could have a direct effect on the permeability of the plasmalemma and hence the supply of substrates locally for its own growth - an attribute of importance in the highly competitive environment of the rhizosphere. For example this could be achieved by hormone production. Also Katou et al. (1982) have found a component of hyphal cell walls of Phytophthora infestans can affect the membrane potential of potato tuber cells.

Much has been written over recent years about exudates and correlations between exudates and VAM infection under various conditions and about soluble carbohydrates in roots and VAM infection (see Bowen, 1984). Often the correlations are not extremely good and often they are better with the length of root infected than with numbers of infections.

Schwab et al (1983a) and Robson et al (1984) have recorded greater external growth of hyphae in phosphate deficient soils. It is appropriate to examine the sources of substrates from roots driving fungus growth in the rhizosphere and into soil. Substrates in the rhizosphere, often loosely referred to as 'exudates', have several different sources (Rovira et al. 1979) ranging from selective leakage from intact epidermal cells to lysates of senescing cells (which are common on most parts of the root). "Exudates", as usually collected, thus have a mixed origin and come from several parts of the root. Nevertheless, true exudates (and lysates to some extent) reflect the composition of the host cell cytoplasm. However, the substances available to the arbuscules in infected cells also come from the host cytoplasm, perhaps modified from uninfected cells because of the increased metabolism in the cytoplasm with arbuscule formation. To study rhizosphere growth in the absence of infection is indeed one of the few ways (perhaps the only unequivocal way) to assess the importance of root exudates to external growth relative to the importance of translocation of substrates from the arbuscule. There is no doubting some importance of root exudates for growth of the hyphae in the rhizosphere, for VA endophytes grow in the rhizosphere of non-host plants i.e. in the absence of infection.

If fungal spread along the root and into soil is driven by energy (and growth factors) derived from the arbuscule, what determines the rate of growth and the restriction on growth of each infection unit (Walker and Smith, 1984) and what determines the longevity of arbuscules? The simplest theory would be to relate both of these to availability of substrate (or growth factors) from the host cell. Until we know more about the substrate preferences of VA endophytes it is unwise to be too dogmatic in conclusions drawn from analyses of extracts of roots and exudates. However if we knew more about the substrates used by VA endophytes we might be in a better position to understand spread of the infection and longevity of arbuscules and to relate these to root physiology and the effects of environment on cytoplasm composition along the root. Until then, it might be worthwhile to have some study of distribution of translocated assimilate (regardless of identity) along the root in relation to mycorrhizal growth and arbuscule longevity. For example, Rovira and Bowen (1973) showed that there was quite a different distribution of translocated assimilate along wheat roots at different soil temperatures: 24 hr after giving a pulse of $^{14}CO_2$ to the tops the portion of the root 6-14 cm behind the apex had only 20% of the translocated assimilated at 20°C soil temperature but at 10°C this figure was 30% and at 5°C it was 47%. If the substrate theory is correct, we could expect greater arbuscule longevity in sub-apical portions of the root, with lower soil temperature.

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CLASSIFICATION, EVOLUTION AND TAXONOMY OF MYCORRHIZAE

CLASSIFICATION OF ECTOMYCORRHIZAE: WHAT'S NEW AND WHAT TO DO

By

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Keywords: Description, morpho-structural series.

INTRODUCTION

One of the first steps in the process of knowledge is the ability to identity the object under study. Since Frank, in 1885, the identification of the partners forming the ectomycorrhizal association is still not possible, with fewexceptions. It is mostly the fungal symbiont which needs to be identified as it is usually not a problem to determine to which host plant belong the given ectomycorrhizae. The classification attempts should then focus on the fungus taking into accounts the new developments in the classification of higher fungi. The task is not a simple one as several thousands species of ectomycorrhizal fungi are involved.

Mainly the new insights in the classification of ectomycorrhizae will be stressed here, Zak (1973) having made an excellent review on this subject. Four main subjects will be treated: 1- Why a classification of ectomycorrhizae is needed? 2- New classification concepts of ectomycorrhizae. 3- The morpho-structural series in ectomycorrhizae. 4- Characterization of ectomycorrhizae. The ideas expressed here in ectomycorrhizae may be applied to other root-fungi associations such as ectendomycorrhizae and endomycorrhizae.

1. Why a classification of ectomycorrhizae is needed?

The advantage of the classification of ectomycorrhizae is that it provides us a tool for the identification of the fungal symbiont of a given ectomycorrhiza. A practical system permitting such an identification would open new insights into the distribution and the ecology of these fungi. With few exceptions, the taxonomy of higher fungi is based entirely on the fruiting body. Without fruiting bodies, no identification is possible. Very little is known about the fruiting behaviour of ectomycorrhizal fungi. As Molina and Trappe (1982) recalled, "the ectomycorrhizal host potential of fungal symbionts may differ strongly from that observed simply from sporocarp-host associations". A classification of ectomycorrhizae would allow the study of ectomycorrhizal fungi in a more accurate way. The host plant range and the geographical distribution of a given ectomycorrhizal fungi should be set in a better way. Population studies of these fungi and their ecology would become more accurate than studies based only on the sporocarp. Such a classification would also found applications world wide in inoculation programs.

Moreover, a classification by itself would lead to a better taxonomy of higher fungi. Additional characters would be available to improve the natural classification based only on the sporocarp. This has been the case for the genus Byssoporia (Larsen and Zak, 1978). It seems also probable to rely on the existing morpho-structural series observed in ectomycorrhizae (see section 3) to determine into some extent the evolutionary state of a fungus.

2. New classification concepts in ectomycorrhizae.

The classification attempts of ectomycorrhizae were done mainly to survey the range of morphologies and structures of ectomycorrhizae without knowing the specific fungi involved. Groups of ectomycorrhizae were defined as if the ectomycorrhiza was a living organism by itself, like a lichen. The results were an artificial grouping unrelated to the taxonomy of higher fungi. In fact, the ectomycorrhiza belongs to a plant and is only a part of it. The fungus is the "added" organism and is mainly responsible for the observed morphology of ectomycorrhizal roots. A classification of ectomycorrhizae should be considered in our opinion as a classification of ectomycorrhizal fungi based on their vegetative state e.g. on their non-reproductive structures. It is more a classification of fungi than of "ectomycorrhizae".

Since Dominik (1969), no other classification schemes have to our knowledge been offered. Numerous papers have offered descriptions, most of the time incomplete, of known host-fungus ectomycorrhizae but with no attempts made to build a taxonomically related classification system of ectomycorrhizae.

Voiry (1981) has proposed a gross classification system based on morpho-structural characters of natural and synthesized ectomycorrhizae of beech and oak. This scheme was somewhat based on the genera of fungi involved. An important step was made in associating a fungus genus to a constant ectomycorrhizal pattern. Mason and his coworkers also recognized to some extent their type of ectomycorrhizae by the fungus genus (e.g. Hebeloma-type of ectomycorrhizae). Godbout and Fortin (unpublished) independently arrived at similar conclusions to that of Voiry (1981) from synthesized ectomycorrhizae of aspen.

This means that it is possible to identify distinctive characters of the fungus in ectomycorrhizae that will allow the same grouping as the one based on sporocarp characters e.g. the actual taxonomy of higher fungi. Distinct ectomycorrhizal characters are very likely to be found in all levels such as families, genera, subgenera, species. For example, vegetative features very likely shared by all the fungi belonging to the Cortinariaceae are the woolly mycorrhizae, the loose mantle outer layer and the well-developed extramatrical phase with hyphae bearing clamp connections. Russulaceae, on the other hand, very likely form smooth mycorrhizae having a compact mantle, a poorly-developed extramatrical phase and hyphae without clamp connections. The genus Lactarius is reported to possess mycorrhizae with lacticifers, Russula to possess cystidia on the surface of the mantle, Leccinum to have beaded hyphae in the Hartig net, etc. Within the genus Amanita, we have found structural differences in the mantle that follow the actual subdivision of the genus (unpublished). Much more work is needed to define the constant and distinctive characters of a given group of fungi. Such

studies should be undertaken on one genus at a time in order to set the ectomycorrhizal characters of the genus. Once the genus characteristics are defined, a key to the species can be built along with groupings in subgenera and/or sections. It is not sure that it will always be possible to separate ectomycorrhizae at the species level on a morpho-structural basis. Chemical analysis would probably then be necessary.

This classification system relies on the modern taxonomy of higher fungi and will provides additional data for a more natural grouping. Delimitations of a genus and of its subdivisions are very likely to benefit from such a characterization. Mycologists should undertake a large part of the classification of ectomycorrhizae. They are well prepared for a study in which the taxonomy of ectomycorrhizal fungi is involved.

Very likely, only a single identification key to the ectomycorrhizal fungus symbionts will have to be built for all host plants. Several papers reported that similar ectomycorrhizal patterns were formed by a specific fungus on several host plants. The well known ectomycorrhizae of Cenococcum geophilum Fr. and Piloderma croceum Erikss. & Hjortst are striking examples. This fact however remains to be demonstrated and must rely only on the fungal tissue and not on the host tissues!

3. The morpho-structural series in ectomycorrhizae.

Voiry (1981) proposed somewhat natural classification key based on two major characters: the extramatricial phase organisation and the mantle structure. From the available data in the literature, some fungus genera can be plotted along these two characters on a graph. (Fig. 1).

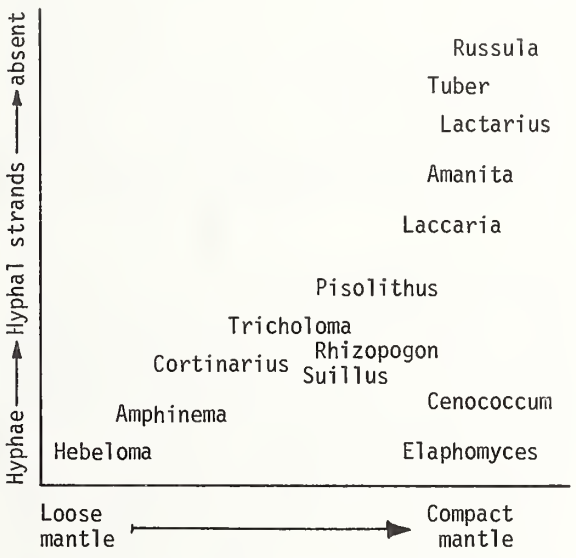

Fig.1 Position of some genera of fungi in the morpho-structural series.

From this graphic, some combinations of these two characters seem not to be found or are rare in ectomycorrhizae. A loose mantle with only hyphal strands or without an extramatrical phase seems not to exist. A synenchyma with abundant extramatrical hyphae is rare. These structures were not mentioned by Dominik (1969). This graphic shows also that the Basidiomycetes are located mainly from bottom-left to upper-right, Ascomycetes mainly bottom-right to upper-right.

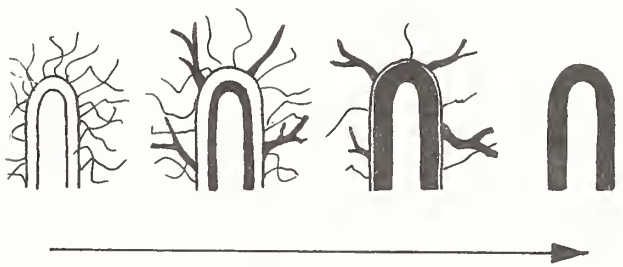

Fig. 2 The morpho-structural series.

Figure 2 illustrate the sequence in the structuration of ectomycorrhizae. It goes from a loose mantle with only hyphae to a compact mantle without (or peridiodically with) an extramatrical phase. The fungal tissue structuration increase from left to right in this figure and from an upward, left to right direction. This organization process is followed also by a specialization toward the roots. The "substrate" of the fungus is much more the root in Russula where nearly all the hyphae are tightly appressed around the root, than in Hebeloma where the hyphae are well dispersed in the soil and loosely interwoven around the root. Concurrently the mantle hyphae become mainly isodiametric and the fungus influences the root toward a more branched habit. Ectomycorrhizae at the upper right very likely represent a closer association between the fungus and the plant than those of the bottom left in figure 1.

A better ordering of the fungus genera could be achieved with additional characters such as clamp connections, mantle thickness, shape of the mantle cells, degree of ectomycorrhiza branching. Such available data would obviously bring a clearer understanding of the genera position in the series. It should be mentioned that the position of a given genus occupies an area rather than a point in this ordering as the species within a genus are also ordered in a similar way. That is, the species within a genus could also be positioned into a morpho-structural series.

4. Characterization of ectomycorrhizae.

The most widely applied method for characterizing ectomycorrhizae has been a morphological and structural one. Chemical techniques including immunofluorescence, electrophoresis, pyrolysis gas chromatography, pigment chromatography, fluorescence and macrochemical reactions, have also been tried. Some of these techniques may become very useful if not essential for the identification of the fungal symbiont at the species level. The morpho-structural way of characterizing ectomycorrhizae will be essentially treated in the

following lines.

Descriptions of ectomycorrhizae should emphasize the fungal tissues as these probably remain constant for a given fungus whatever the host plant, as does the sporocarp. The structural organization of the fungal tissues is of prime importance in characterizing ectomycorrhizae. Hyphal strands possess a variety of structures as does the mantle and the Hartig net. Each description should illustrate the mantle structure in section and in plan view. A great attention should be given to the possible presence of specialized cells such as cystidia, setae, laticifers, gelatinized hyphae, etc. These cells are very distrinctive and should be well documented. A character which has been overlooked is the color opacity of the mantle. When the hyphae are hyaline, or translucent-colored, the color of the ectomycorrhiza is close to that of the root, that is whitish when young becoming brownish when old. The translucent state of the mantle can be recognized by looking at the side of the ectomycorrhiza where only the mantle is present. Mantles can be "hygrophanous" as they are translucent when water-soaked and opaque as they become dry. The degree of branching of an ectomycorrhiza is very likely indicative of some fungal hormone production and seems characteristic of the fungus. The pattern of branching, on the other hand, is a host character.

Microscopic characters should be emphasized over macroscopic ones as the former remain constant whatever the soil compactness or moisture content. Microscopic characters are also more numerous. Macroscopic characters are mostly intended for the gross sorting out and grouping of "similar" ectomycorrhizae. The same procedure can be observed for higher fungi identification in modern taxonomy. Semi-thin sections (about 1 µm) are almost a must for radial or longitudinal characterization of the mantle and the Hartig net because of the high degree of resolution. Descriptions of ectomycorrhizae should invariably include pictures and/or precise drawings of the various structures encountered. An image is worth a thousand words! No precise vocabulary is available to describe accurately the observed structures. Such a clear and precise vocabulary is awaited and illustrations should be included for ectomycorrhizal characterization.

The material chosen for descriptive studies would ideally be from field-collected ectomycorrhizae. Synthesized ectomycorrhizae should be chosen only in media without sugars, as they may induced artefacts in the observed structures. Techniques that allow visual observations of the development of the extramatrical phase and formation of ectomycorrhizae should be preferred against others.

Collections of ectomycorrhizae of known fungus and host plant should be secured and placed in an herbarium. Classification works should be based on available material which would save invaluable time. Collections should be kept dried and in a fixative like glutaraldehyde for an all purpose characterization. Notes and pictures should be taken if possible.

In conclusion, the classification of ectomycorrhizae is a serious task and it is not with casual descriptions made by one and another that progress will be made. Studies must be performed with the purpose of classification and integrated into such a framework. Nearly all has to be done in this field to ensure a useful and accurate identification of the ectomycorrhizal symbionts.

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EVOLUTION OF MYCORRHIZAE

By

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Keywords--Boletes, co-evolution, conifers, dicots, ectomycorrhizae, fine endophyte, VAM, Acaulospora, Boletus, Endogone, Entrophospora, Gigaspora, Glomus, Leccinum, Microcodium, Palaeomyces, Palaeosclerotium, Rhizophagites, Sclerocystis, Suillus, Tomentella

Introduction

The first mycorrhizal symbioses appear to have occurred early in geological time under conditions not clear to us today. In spite of an increasing knowledge of fungal and plant fossil history and mycorrhizal symbioses we will never be sure of the earliest history of the organisms involved and must be content with informed speculation. We must also recognize that not all mycorrhizae have a common origin and that these symbioses have arisen a number of times since the appearance of the first land plants.

Our approach in this session is clearly that of mycologists and we have divided our task as mycologists would. Because of time limits we have focussed our attention on only two types of mycorrhizae, ectomycorrhizae and vesicular- arbuscular mycorrhizae, but recognize that these are but two of several kinds.

Ectomycorrhizae (O.K.M. & H.D.T.)

Ectomycorrhizae, probably the most recently evolved type, involve a restricted segment of the plant flora. Several families of present-day plants are ectomycorrhizal, including the Betulaceae, Salicaceae, Fagaceae and Pinaceae in the Northern Hemisphere and Mytraceae and Nothofagus (Fagaceae) in the Southern Hemisphere.

Two lines of evidence appear pertinent to any consideration of the origins of ectomyorrhizae, 1) the early history of the Coniferales and 2) fossil history of the "higher fungi". The Coniferales seem to have arisen from mid-Palaeozoic Corditales, which in turn find their origins in the Progymnospermopsida in the Devonian. Members of the Coniferales developed slowly during the Jurassic and Triassic periods. Only one family, the Pinaceae, is ectomycorrhizal but it is exclusively so. Most of the fungi that are known to form ectomycorrhizae with Pinaceae today are Basidiomycetes and the report by Dennis (1969) of hyphae with clamp connections in the wood of the coenopterid fern Zygopteris illinoiensis is thus significant. This discovery indicates a wood-decay fungus with dikaryotic cells and typical basidiomycetous clamp connections as early as the mid-Paleozoic. If we assume that mycotrophy evolved prior to the proliferation of substrate-specific decomposers then we should look for the development of early ectomycorrhizal Ascomycetes and Basidiomycetes no later than the middle Pennsylvanian period.

An early mycorrhizal fungus would have had to withstand wide variation in temperature, soil pH, low moisture content, high UV radiation, and be able to exist in the "soil" for long periods of time. Fungi resembling Palaeosclerotium Rothwell seem to possess such characteristics as far as we can tell. Dennis (1976) described and illustrated a similar fungus with a simple ovoid ascus borne within a pseudoparenchymatous stroma. The ascospores are spiny and resemble those of many present-day soil fungi. Such early sclerotial forms bear a striking resemblence to Cenococcum geophilum Fr. and the Cenococcum-like anamorphs common in most ectomycorrhizal communities today. Present-day anamorphs referrable to Cenococcum produce abundant sclerotia, similar to those of Palaeosclerotium pusillum Rothwell and are tolerant to drought, low soil pH and other environmental stresses.

Dennis (1976) also illustrated indisputable clamp connections with his ascomycetous fungus, but there is reason to doubt that they are part of the same fungus. Peterson, Mueller and Englander (1980) recently demonstrated ultrastructurally that what appeared to be a single symbiont in Rhododendron was in fact two fungi, an Ascomycete and a Basidiomycete. These authors concluded that the Rhododendron root fungus relationship is complex and were able to distinguish the two fungi only with electron microscope. Dennis' report may represent a similar phenomenon.

The roots of Rhododendron studied by Peterson, Mueller and Englander were found associated with a Basidiomycete of the genus Clavaria. Circumstantial evidence and field observations suggest its role as a mycorrhizal symbiont. The Clavariaceae is one of the aphyllophoralean families which produce both simple and polymorphic fruiting bodies and it suggests that it is a group in which we might seek a "primitive" ectomycorrhizal Basidiomycete. There are several compelling features possessed by some members of the Corticiaceae, Thelephoraceae and Clavariaceae which suggest an area from which some present-day relations of the earliest ectomycorrhizal Basidiomycetes might have evolved.

Particularly interesting among the Aphyllophorales are some of the simple polymorphic members of the Thelephoraceae, which are cosmopolitan in distribution, have a broad host range and colonize hosts under a variety of conditions. Miller (1982) suggested that Tomentella species may be representative of an early type of mycorrhizal fungus along with the similar type of corticiacean fungi such as Philoderma bicolor. These fungi are very simple in form and consist of a mold-like colony bearing a palisade of basidia. It seems a small step, then, to evolve a small upright Clavaria-like fruiting body from this and attain a more efficient spore-bearing apparatus. From here even more complex types might arise.

The complexity of modern ectomycorrhizal fungi is well-known. Best known are the mushrooms and boletes, which make up most of the ectomycorrhizal fungi. The latter, especially, represent a large group of almost exclusively ectomycorrhizal species. The boletes constitute a group of fleshy putrescent fungi which closely resemble gilled mushrooms except for their tubular hymenophore. They appear to be most abundant and occur in greater variety in the more temperate climate of North America. In temperate regions they are almost exclusively ectomycorrhizal, but in the tropics some taxa appear to be non-mycorrhizal. Mycorrhizal hosts range from some of the conifers to several genera of woody dicots.

The origin of the boletes is obscure, however, it has been suggested that they arose from clavarioid type of ancestor having a smooth hymenophore perhaps similar to the present-day Clavariadelphus. From this ancestral type there was next a stage with a more elaborate hymenophore such as that seen in Gomphus in which the hymenophore is developed in a series of low, irregular obtuse folds. At this evolutionary step perhaps a dichotomy developed with one line leading to the gill fungi and the other to the boletes. In the bolete line further dichotomies might have occurred resulting in a rough-spored line and a smooth-spored one. The sequence of evolutionary stages within the boletes is highly speculative and problematical. The end result is, from a conservative point of view, the formation of from one to fifteen genera.

So far as known, boletes form mycorrhizal associations only with woody taxa belonging to conifers in the Gymnospermeae and to the dicots within the Angiospermeae. In the coniferous group these associations are apparently restricted to members of the Pinaceae and in the Angiospermeae, to a large extent, to the Fagales and Salicales, and to a much lesser extent, to the Myrtales, Ericales, Dipterocarpales and perhaps others.

Within the boletes the genus Suillus, perhaps a primitive group, shows the most interesting mycorrhizal associations. With only one or two exceptions, these fungi are restricted to associations with members of Pinaceae. Some species, particularly on the west coast of North America, are apparently restricted to a single host species while others enjoy a much wider spectrum of hosts. This rather severe restriction might, according to Corner and Singer, represent a derived state and shows a high degree of specialization between the two taxa. However, might this restriction be interpreted as representing a relictual state of what was a more widespread relationship? How can one account for host restriction in these states? Did it result from the production of a unique type of photosynthate or from the elaboration of some other chemical compound, or did it arise from some other cause?

The genus Boletus appears far more advanced than Suillus, is very large and has a wide spectrum of mycorrhizal hosts. Most species are associated with members of the Fagales and Salicales or, to a much lesser extent, with other dicotyledonous plants. In addition, several are associated with members of the Pinaceae. The apparent absence of associations with the more primative woody dicots such as Magnoleae and Guttiferae (with the exception of Shorea) might be of significance regarding the origin not only of the boletes themselves but also of ectomycorrhizal associations as well. Also, does the presence of non-mycorrhizal species in the tropics possibly indicate that mycorrhizal associations, at least within the Boletes, arose in such a region and then migrated to drier more harsh areas where such an association contributed more heavily to the survival of both fungus and vascular plant?

Leccinum constitutes what seems to be the most complex taxon of boletes. It appears to be of recent origin, so much so that there is still considerable integration among species, thereby making positive identification of the species very difficult. Mycorrhizal associations within this group seem to occur most commonly with members of the Betulaceae and Salicaceae. Other families involved to a lesser extent include the Fagaceae, Myrtaceae, Ericaceae and Pinaceae. Because of the uncertainty associated with the correct identification of species, the determination of definitive mycorrhizal associations remains somewhat tenuous at present.

The rough-spored genera of boletes, exemplified by Strobilomyces and Boletellus seem more elaborate in their specification in the tropics and subtropics. They are far more common in the warmer more humid Gulf Coastal Plain than elsewhere in the United States. It is surprising that they are completely absent in this country west of the Rocky Mountains. If, as postulated by Corner (1972), they are a primitive group then their tropical affinities might also point to a tropical origin of these fungi and since, so far as is known, they are mostly ectomycorrhizal, it might also indicate a possible sight of origin of ectomycorrhizal association.

Vesicular-arbuscular mycorrhizae (S.M.B.)

Vesicular-arbuscular mycorrhizae (VAM) are formed by the vast majority of land plants; exceptions include Pinaceae, Betulaceae, Fagaceae, Salicaceae, Cruciferae, Orchidaceae, and Ericales. Most VAM fungi form isolated spores on fragile hyphae in their phytosymbionts' rhizospheres which makes determining host association in the field rather difficult. VAM fungi have not yet been grown in axenic culture which complicates synthesis experiments to determine in vitro host specificity. For these reasons, host range of VAM fungi remains virtually unknown. Because of the relatively recent discovery of their identity, little is known of the geographic distribution of VAM fungi, particularly when compared to larger, epigeous ectomycorrhizal fungi. Our ignorance of host range and geographic distribution of these fungi makes consideraton of their evolutionary origins a matter of pure conjecture. Nonetheless.....

Subterranean organs of early land plants have been found to contain hyphae and vesicles that closely resemble those of modern Glomus mycorrhizae (Kidston and Lang 1921), although there has been some debate over their nature: mycorrhizal or saprophytic (Taylor 1981). Colonization pattern and vesicle morphology of modern Acaulospora and Entrophospora mycorrhizae may distinguish them from Glomus and Sclerocystis mycorrhizae. In Gigaspora, intracortical vesicles are few and borne on spiralled hyphae. Vesicles of "fine endophytes" are much smaller than those of other taxa. Hypha diameter and pattern of branching and colonization also differentiate "fine endophytes" from other VAM fungi. Assuming that mycorrhiza morphology is stable and that no major extinction of VAM fungi has occured, it may be possible to identify genera of fossil fungi based only on their VAM and thereby establish when the various VAM fungi appeared. Similarly, generic identity of fungi forming VAM of modern plants may be determinable if mycorrhiza morphology is examined more closely.

Fossilized Glomus and Sclerocystis spores are common in preserved soils and roots, though they have seldom been recognized as such and therefore received a number of different names. Microcodium elegans Glück is a name that has been applied to fossils variously interpreted as green algae (Glück 1912), coral (Capeder 1904), and ectomycorrhizae (Klappa 1978). When Wood and Basson (1972) reported finding them in Paleozoic shales of Missouri, they were actually describing and illustrating remarkably well-preserved sporocarps of a Sclerocystis species, probably S. rubiformis. Rhizophagites Rosendahl was recognized by Gerdemann and Trappe (1974) as being fossilized spores of Glomus species. Wagner and Taylor (1981) reported Glomus-like spores from tissues of Pennsylvanian aged fossil plants. Apparently, no other modern VAM genera have been reported, possibly because they are not represented in the fossil record, but probably because they have not been recognized. The real and potential existence of such a fossil record means that in this group of fungi classical evolutionary study may be possible.

If one accepts, for the moment, the hypothesis that VAM fungi co-evolved with land plants (Pirozynski and Malloch 1975), to understand their early development it is necessary to imagine the environment that these fungi would have encountered. Beginning in the late Precambrian, soils were organically modified by algae, bacteria, viruses, and perhaps liverworts adapted to surviving periodic drying. It is possible that saprophytic or lichenized fungi were also present by this time (Taylor 1981). Land plants began to develop either in the late Ordovician (Gray and Boucot 1977) or late Silurian (Edwards et al. 1979), and by the Devonian there is good evidence that fungi resembling modern VAM already colonized their rhizomes and roots. The major question at this point must be whether the origin of VAM fungi lies in decomposers already established in accumulating plant debris, in parasites of predecessors of vascular plants, or somewhere else. In fact, this question repeats itself if VAM arose more than once from independant origins, as suggested by the diversity of their spore and mycorrhiza morphology. If, as Retallack (1981) suggested, holdfasts, rhizoids, rhizomes and roots of primitive plants have been preserved in aquatic shales and cherts, they may bear evidence of ancestors of VAM fungi. If VAM fungi arose directly from parasites of these aquatic or semi-aquatic plants, VAM fungi would have undergone changes as their plant partners also adapted to a strictly terrestrial environment.

An alternative hypothesis is that Palaeomyces and other early fungi resembling VAM were in fact saprophytes. Fungi existing in early organically-modified soils as decomposers of plant material, might already have had chitinized walls, as do modern VAM fungi (Weijman and Meuzelaar 1979), providing a resistant barrier to fluctuations of temperature, pH, moisture, and UV radiation. The zygosporic Endogone pisiformis (Endogonaceae) forms, in roots and other tissues of dead plants, hyphae and vesicles that closely resemble those of VAM (Berch and Fortin 1983). Other than the obviously zygosporic nature of E. pisiformis, there are many morphological similarities between this fungus and modern Glomus species. One striking similarity between Endogone and VAM fungi, that distinguishes them from other zygomycetes, is their lack of sporangia. (In passing, I note that ornamented vesicles of Gigaspora spp. ressemble reduced sporangia.) If VAM fungi are related to zygomycetes they appeared early in the evolution of these fungi. Perhaps aerially dispersed sporangiospores were developed later by zygomycetes better adapted to terrestrial life. In changing from a saprophytic to a mutualistic association with plants, sexual recombination would have been detrimental to a zygospore-forming organism, perhaps similar to E. pisiformis, since it would give rise to progeny less adapted to mutually beneficial association with living plants.

VAM is the most common type of mycorrhizal association in areas of tropical and temperate vegetation, but in the boreal forest there is a switch to predominantly ectomycorrhizae. This suggests that the center of evolution and distribution of the VAM fungi has been the tropics, but since it has not been determined whether specific VAM taxa are found primarily in particular climatic or vegetation zones, this remains an untested hypothesis. If VAM fungi co-evolved with early land plants, it is reasonable to suggest that their early environment was tropical.

Presently all of the VAM fungi are classified in the Endogonaceae, Zygomycotina (Gerdemann and Trappe 1974), even though none of them form either zygospores or sporangiospores. I believe that, in the VAM fungi, generic differences in morphology and development of spores and mycorrhizae reflect independant evolution of a shared nutritional habit from a number of distinct points of origin. I recogrize four distinct VAM groups:

1. Glomus - Sclerocystis
2. Acaulospora - Entrophospora
3. Gigaspora
4. Fine endophyte

Over the last ten years, the number of described species of known or probable VAM-forming fungi has gone from 30 to over 100. As species numbers and variability are determined, generic concepts will change. With a shift in interest in these fungi from practical to fundamental, many of the questions raised here will be addressed and the data base for taxonomic revisions will become more extensive. The midst of a period of active species cataloging is an inappropriate time to propose major systematic change, but such change is inevitable.

This discussion of evolution of the VAM fungi is intended to pose questions that can be answered through further research: What is the geographic distribution of modern VAM fungi? Do modern VAM fungi show host or environmental specificity? Can fossilized spores of VAM fungi be identified? Are spores of all genera of modern VAM fungi represented in the fossil record? How diagnostic is mycorrhiza morphology? Do modern or ancient aquatic plants harbour fungi related to VAM fungi? How are VAM fungi related to zygomycetes, other fungi, and to each other?

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TAXONOMY OF THE ENDOGONACEAE

By

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Keywords - *Acaulospora, Complexipes, Endogone, Entrophospora, Glaziella, Gigaspora, Glomus, Sclerocystis*

Introduction

In the past decade, there has been a large increase in the number of descriptions of species in the Endogonaceae, and an enormous amount of research into the effects some of these fungi have on plant growth. The proliferation of species and the variation in the standards of descriptions (ranging from a complete lack of formal description to full and detailed discussion of the species morphology and how it differs from other similar species) has inevitably caused much uncertainty in the mind of the scientist who may simply desire to have a name to attach to an experimental organism but who does not desire to become involved with taxonomy.

Unfortunately, identification of these fungi is extremely difficult, partly because the taxonomic concepts are not yet fully developed, but also because only a fraction of the species likely to be encountered have been described. These difficulties have led to considerable confusion and this undoubtedly has resulted in the same specific epithet being applied to more than one taxon. The practice of passing round misidentified cultures that rarely are checked by their recipients has also caused problems.

As the number of new species threatens to outnumber the characteristics that can be used to separate them, the time seems ripe for a careful reconsideration of both the taxonomic concepts used in the group, and of the general attitude to the identification, classification, and preservation of fungi used in experiments with vesicular arbuscular or arbuscular mycorrhizae.

Smith (1980) stated that the "... study of mycorrhizal plants has now progressed from *determination of the fungi involved* (my emphasis) ... to consideration of the mechanisms by which mineral nutrients are absorbed ..." It is my contention that no such position of knowledge exists. We know only a few of the fungi that might form endomycorrhizae. Almost every sample of soil I have examined from Great Britain contains some undescribed species of endogonaceous fungus. It is doubtful if the time will ever arrive when there no longer is a need for the study of the taxonomy and systematics of these important fungi.

The need for taxonomy

It should hardly be necessary to defend taxonomy as a science, any more than it is necessary to defend the study of physiology or ecology of organisms. Yet I am frequently called upon to justify the "need for giving everything names", and there seems to have been little change of attitude amongst mycorrhizasts since Gerdemann and Trappe (1975) decried the irritation displayed by the non-taxonomist towards the progress made in the taxonomy of the vesicular arbuscular mycorrhizal fungi. The need for classification should be evident enough. If I, as a forester, were simply to state that I was planting trees, I would very soon be asked "what species?" Before I can work successfully with these organisms of my trade, I must learn the silvicultural requirements of the different species (and, indeed, of the provenances within these species), so I can apply this knowledge to their selection for large-scale forestry, or, for that matter, for research. The same argument applies to the fungi that form mycorrhizae.

Recognition of taxonomic groupings is fundamental to all science. Without an adequate way of identifying, classifying, and cataloging the species with which we work, the results of our research will be diminished in value, and will be less amenable to interpretation and extrapolation. Conflicting results reported in the literature may be due not to real differences within a taxon but to interspecific variation, the species used having been misidentified and given a common, but incorrect, name.

It is the aim of the taxonomist to sort and synthesize all known information on an organism and to make comparisons with other, similar entities, with a view to bringing some order to the confusion caused by vernacular names and arbitrary classifications. The purpose is to help, not to hinder, other workers. If the rules of Botanical Nomenclature are scrupulously followed, then it will always be possible to reexamine material at a later date as taxonomic concepts change.

The present grouping

The history of the taxonomy of this group (the work principally of Thaxter (1922), Nicolson & Gerdemann (1968) and Gerdemann & Trappe (1974)) was related at a symposium at Leeds University in 1974 (Gerdemann & Trappe 1975), and no useful purpose will be served by reiterating it here. The current ordinal grouping has formalized the order Endogonales possessing a single family, the Endogonaceae, in the Zygomycetes (Benjamin 1979). Seven genera are included (Trappe & Schenck 1982). An eighth genus, *Complexipes* (Walker 1979) was originally tentatively placed in the group. The wisdom of stressing the tentative nature of this placing, both in title and text, was vindicated when the species was later shown to be an ascomycete anamorph, and the name should be applied to sporulating forms of the so-called "E-strain" fungi (Danielson 1982). The basic generic concepts in the family are described and illustrated in Trappe (1982) and Trappe & Schenck (1982). The genera now included are discussed below. Some of what I have to say about them will be controversial, but I have been specifically asked not to eschew controversy in this presentation. Besides being controversial, I intend also to be speculative, and no doubt some, if not all, of my ideas will be proven incorrect in the future. If my

comments serve only to stimulate further research then my purpose will have been achieved.

ENDOGONE

This is the type genus of the family. The spores apparently are zygospores, and are formed in compact sporocarps. Some species form ecto-mycorrhizae, of a sort, with certain trees, while the nutritional mode of the majority is unknown. As far as I am aware, no indisputable evidence of karyogamy or meiosis exists. I understand that one species has now been induced to sporulate in gnotobiotic culture, and perhaps new evidence will soon be forthcoming (S. M. Berch, pers. comm.). In view of the differences in spore formation between this genus and the others presently in the family, and of the failure of *Endogone* spp. to form endo-mycorrhizae, it seems to me that this should be the sole genus in the monogeneric family Endogonaceae.

GLAZIELLA

Only once have I had the opportunity to examine a specimen of *Glaziella aurantiaca* (Berk. & Curtis) Cooke, the sole member of this genus. I was given a small portion of a sporocarp from the collection of Dr. J. W. Gerdemann. From such a small sample, it is impossible to give a considered opinion. Probably the species should be in a different family from *Endogone*, since it does not seem to be zygosporic. It is not known if it forms any sort of mycorrhizal association, and until further study is made of fresh material, including attempts to induce symbiosis, its real position will remain unclear. Some of the participants at this conference may have the opportunity to collect and study this tropical fungus, and I would encourage them to do so. I welcome receipt of specimens of this fungus, or of any other member of the Endogonaceae.

ACAULOSPORA, ENTROPHOSPORA and *GIGASPORA*

Acaulospora and *Entrophospora* both form their spores in a similar way, from a sporiferous saccule (Walker et al. 1984), and both form vesicular arbuscular mycorrhizae. Each of these genera is distinctive, and with the information we have from presently described species, they seem unlikely to be split further. There is a suggestion that the former genus may have a phase producing spores of a similar nature to those of *Glomus*, though I have not seen material to confirm this. I would require considerable evidence to convince me that this indicated phylogenetic linkage between the two genera.

Gigaspora is not a discrete grouping, and should be separated into two distinct genera based on wall structure, form of auxiliary cell ornamentation, and germination characteristics (Walker & Sanders 1984). Those authors propose to erect a new genus, *Scutellospora*(?), in which to place those species that germinate by way of a germination shield. The apparent similarity of the chambers formed in a germination shield to the suspensor cells of some zygomycetes stimulates my thoughts on the true nature of the spores. Study of the cytology of these spores would, I believe, be particularly rewarding.

These genera form an apparently natural group. I am aware of no evidence to link *Entrophospora* with *Gigaspora*, but the latter is clearly closely related to *Acaulospora* by spore morphology and germination characteristics, and also by the production of a hyphal reaction to damage, known as *wound healing* (Gerdemann 1955, Walker et al. 1984). The structure of the mycorrhizae produced by fungi in *Gigaspora* indicates quite a large difference between it and the other two genera in this group. Whereas the latter form typical vesicular arbuscular mycorrhizae, the former does not seem to produce vesicles.

It seems to me that this group of genera does not really fall comfortably into the Endogonaceae sensu stricta, and would be better placed in a separate family. Whether they should remain within the Endogonales would depend upon the results of detailed comparisons which are urgently required.

GLOMUS and *SCLEROCYSTIS*

These two genera form a distinct natural grouping, the only distinct difference between them being that *Sclerocystis* spores are formed in an orderly manner around a sterile central hyphal plexus, whereas the spores of *Glomus*, when formed in sporocarps, are less ordered.

The genus *Glomus* is not very clearly defined, and can be separated into a group that germinates through the spore wall, and one that germinates through the subtending hypha. Walker & Rhodes (1981) discussed the generic concepts in relation to germination characteristics, and concluded that it would be unwise to use them to split the genus. Until further evidence is forthcoming, I see no reason to amend this view. Species in *Glomus* seem to be separable by another criterion. Some have a membranous inner wall, while others have no such structure. I have a number of the former awaiting more detailed study and description.

I am ill at ease with the inclusion of *Rhizophagus tenuis* Greenall in the genus *Glomus*, but since I have not studied it in detail, I can offer no better placing. Certainly the mycorrhizae formed by this species are very different from those found in symbioses formed by other species of *Glomus*, and the chlamydo-spore-like structures associated with it are very small in comparison with the average for the genus.

Considerable further study is required before an opinion can be expressed on whether or not there is a need to split the genus *Glomus*, but it seems improbable that it will remain intact in its present form.

It seems unlikely that the separation between *Glomus* and *Sclerocystis* will be maintained. Species from both genera are known to form similar vesicular arbuscular mycorrhizae, and

their spores, when separated from sporocarps, are indistinguishable. As they exist currently, these genera probably should be placed together in a separate family, and their ordinal placing should be reviewed.

Infrageneric taxonomic concepts

In *Endogone*, *Glomus*, and *Sclerocystis*, the structure of the sporocarp is important in delimiting species, but among all genera in the Endogonaceae, the most important criterion in use is spore morphology. There is an excellent synthesis of most of the current concepts in Trappe (1982). Generally, there are sufficient characteristics available to separate the species, but as more undescribed taxa are discovered, it will become increasingly difficult to separate closely related organisms. I have already implied that the present classification is artificial, and it would be best to consider the Gerdemann & Trappe system to be a "form family." This is not to suggest that it is without merit. Indeed, quite the contrary is true. It is extremely valuable as a basis for further study and is, in practice, extremely useful for cataloging the many different taxa. The publication of Mycologia Memoir No. 5 was the major milestone in the recent history of the family, and will be the basis of its classification for many years to come. The synoptic key of Trappe (1982) represents a major step forward in aids for the identification of the species, but it can only help with those species already described, and it is, of course, constrained by the accuracy of the original descriptions from which it was created. There is a tendency to try to force a specimen into one of the existing species, rather than to accept the possibility that an undescribed taxon may be at hand. There is simply no substitute for detailed study of the literature, and even when a specimen has been placed in what is believed to be the correct taxon, it is necessary to refer back to the original descriptions for confirmation of identity.

Even the species that are included in the keys may not be easy to identify. In some instances, the original description was poor, and, as taxonomic concepts have changed, important details have been found to be missing. Indeed, some species bear little resemblance to their descriptions. For example, I have carefully examined the type material of *Glomus mosseae* (Nicol. & Gerde.) Gerd. & Trappe, and, apart from the fact that it contained two species, the spores have an outer evanescent wall layer not even mentioned in the protologue. The lectotype of *G. fasciculatum* (Thaxter sensu Gerd.) Gerd. & Trappe is similar and I will return to the problem of that species later. Too much reliance should not be placed on early descriptions. Careful reexaminatin and redescription of species is needed from time to time to accommodate advances in taxonomic concepts. For this reason, type material should be generous, and widely distributed in herbaria that are prepared to accommodate requests for loans of specimens. The herbaria in some Asian countries have not even acknowledged my requests for loan of type material.

One of the main difficulties is the lack of agreement among authors as to which characteristics are most important, and how to define them so that they are understood by all. Ambiguities have slipped into the literature, and no generally accepted standard seems to exist. I have proposed what I believe to be a suitable terminology for the wall structure of spores as the beginnings of a more standardized conceptual basis for species descriptions (Walker 1983), and have found acceptance among other writers. Since then, a fifth wall type, the leathery wall, has been recognised, and will soon be described in detail.

From time to time, people have proposed that there are non-sporulating species of endogonaceous fungi. This suggestion has been induced by failure to find spores in, usually very limited, field samples. I have to confess surprise at this, as I cannot remember ever taking a soil sample in which no spores at all were present. Absence of the spore type does not necessarily imply absence of the fungus. Samples are rarely taken over extended time periods, and extraction methods often are deficient. I have found that at some locations, especially on agricultural land, there are few living spores. However, if a pot culture is started from the soil concerned, within a year some sporulation has usually taken place in the pots. Some of the "vesicles" found in mycorrhizae are probably spores, rather than vesicles. The species *G. intraradices* Schenck & Smith is not alone in producing spores in the roots of plants and if such species predominate in a location, soil samples are unlikely to contain large number of free spores.

Less conventional taxonomic methods

The beginnings of work on other aspects of taxonomy have been laid. Daniels & Duff (1978) examined differences in germination characteristics of different isolates of *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe. Abbott & Robson (1979) and Abbott (1982) have started the process of characterizing the mycorrhizae formed by different species of fungi. This start of the "whole fungus" approach is laudable, but besides illustration of the mycorrhizae, I would like to see equally good illustration of the spores of the fungus used. The so-called "E-3" organism is described in Abbott (1982) as a form of *G. fasciculatum* and comparisons made with another "strain" of the "same species". While accepting that the "E-3" organism is a distinct taxon, it is undescribed, and most certainly is NOT *G. fasciculatum*. I therefore wonder a little at the comparison that was made. I hasten to add that I am not criticizing the concept of the usefulness of mycorrhizae as a taxonomic criterion, but I would prefer to see illustration of both spores and mycorrhizae in publications in which these descriptions are made.

The question of dimorphism, or even pleomorphism in these fungi has hardly been raised. It first came to my mind when I repeatedly received pot

cultures not containing the species on the label. Was it always a case of misidentification or contamination, or was it possible that the fungi had more than one morph? After all, this is common enough among other groups, and there is no inherent reason why it should not happen here. I believe that evidence will soon be published indicating that at least two species of *Glomus* do indeed possess two distinctly different spore types. This does not invalidate the use of Latin binomials for each morph - witness the Deuteromycetes - but it does point out the need for careful and detailed studies of the development of endogonaceous species in gnotobiotic conditions from single-spore isolates. The work involved in this is enormous, and it would take a considerable change of attitude by the main funding bodies before sufficient money would be made available.

Much more work is required on study of the hyphal network of Endogonaceae. Very little is known about the hyphal phase between germination and sporulation, except within mycorrhizae, and even this is incompletely understood. Until satisfactory cultural methods are introduced, there will be little progress, but it seems to me that there are endless possibilities for investigations into such phenomena as anastomosis (does it occur only within a taxon, or between taxa? If the latter, are the taxa concerned similar and if so in what respects? If they are not similar, what does this say for our species concepts? Are spores formed from anastomosis of fungi with different spore types, and if so, how do they compare with those of their "parents"?).

Chemotaxonomic methods might be used more. What staining characteristics can be used to help in classification, and what do the reactions mean? For example, *Acaulospora scrobiculata* apparently is characterised by a particular reaction to Melzer's reagent (Trappe 1977). Nevertheless, I have received two different collections of specimens which, in every other respect, fit this taxon, but that have absolutely no reaction to this reagent. Two other areas of investigation into chemotaxonomy have been mentioned in the literature. Isoenzyme studies (Gianinazzi-Pearson & Gianinazzi 1976) may be useful, and histochemical methods (Gianinazzi-Pearson et al. 1981) were used to show that polysaccharides in the hyphal wall of *Glomus tenue* (Greenall) Hall are different from those of some other VAM fungi. Immunological techniques are in their infancy with this family, but show some promise, at least in examining intergeneric relationships (Aldwell et al. 1983, Wilson et al. 1983).

Studies such as these are important, but they require that the organisms used be correctly identified initially. I am not convinced that this has always been done, and reiterate my plea that the fungi concerned be illustrated adequately in the publication, and that a large supply of specimens be lodged in a herbarium for independent confirmation of identity. I would also like to see at least one common isolate included in all studies of this type.

Splitters versus lumpers

There is an age-old problem of the supposed dichotomy between taxonomists who classify things in broad taxa, and those who feel that smaller divisions are necessary. So far, in the Endogonaceae, the species described early have generally been too broad but there recently has been a trend towards narrowing the taxonomic base. This began with the separation of *Endogone* sensu lato into distinctive genera (Gerdemann & Trappe 1974), and has moved to extremely fine separations which can only be detected by experienced personnel.

There is no doubt that many scientists would prefer the ease of identification afforded by extremely broad categories, but as more is learned of the different effects caused by "strains" of one species, it is clear that for meaningful comparisons to be made among endophytes, the "splitter" will have to work first. Even if each "species" in the Endogonaceae represents only part of a true species, the number of these is likely to be relatively small, and while it is possible to combine data from several experiments if the taxa are found to be all the same, it is not possible to separate out data from those in which a "species" has been found in reality to consist of several distinct taxa. It is far better to proceed cautiously towards the aim of careful separation of all entities that are even slightly different, than to be too broad, and discover too late that more than one species was involved.

The *Glomus fasciculatum* problem

Perhaps the taxon that causes most confusion is *Glomus fasciculatum*. A computer search revealed that this taxon was mentioned in over 400 (more than 10%) of all the papers published on mycorrhizae (including all types) in the last decade. Doubtless, since the computer was searching only abstracts, the species was used in many more studies than that. It seems that anything even vaguely resembling a *Glomus*, and roughly in the right size range, is categorized as *Glomus fasciculatum*. Even the "E-3" organism, which clearly is nothing like *G. fasciculatum* usually is classified in this taxon. To some extent, this is understandable. The description in Mycologia Memoir No. 5 is far too broad, and was generated from study of a large number of specimens, some of which probably should have their own specific epithet.

It seems to me that the best thing to do with this species, is to start again from a detailed redescription. I have presented posters describing the species at two conferences, one in Great Britain, and one in the United States. From type material, and living isolates of fungi that are indistinguishable from it, I propose to redescribe the species. A brief summary of the main features of the species follows:

> *Glomus fasciculatum* has chlamydospores borne singly in the soil, or in clusters, or in small sporocarps. Individual spores

are 60-85 x 60-70 µm, possessing a thin, hyaline outer unit wall (wall 1) that usually is less than 1 µm thick and is sometimes very difficult to observe, and a thick, coloured laminated inner wall 6-14 µm thick (wall 2). Mature spores are light yellow to very pale yellow-brown in colour, but are never dark brown or hyaline.

I should emphasize that the size range of spores given here may be subject to amendment when more isolates have been studied. With this caveat in mind, isolates should be examined carefully, and should not be identified as *Glomus fasciculatum* unless they fit this description.

The need for pure isolates

There is a particular need for scientists to pay more attention to the pot cultures they use in research. I am the frequent recipient of requests to identify or to confirm identification of pot cultures. Rarely do I find that these cultures contain the purported species on the label. On one occasion, I examined a culture identified as *Glomus fasciculatum*. It contained thirteen endogonaceous species from among five genera. None of the species in the pot was *G. fasciculatum*! Yet this culture had been used for investigations into growth effects of *G. fasciculatum* mycorrhizae. This is not an isolated occurrence, although it is the most extreme case I have encountered. Recently, a colleague visited me and brought with him three pot cultures obtained from another scientist. All were labelled *G. fasciculatum*, and results from researches with them had been published. My colleague was unhappy with the appearance of the spores, and could not reconcile them with the description of *G. fasciculatum*. He asked if I would confirm the identificaton of the cultures. I discovered that none of the pots contained that species, and that none was a pure culture. Altogether, there were four endogonaceous species among the cultures. The material I examined was from the starter-material sent to my colleague, so contamination after the culture had been passed on could be ruled out.

In another instance, I received a sample of an apparently pure *Glomus* culture. I was careful to examine all the material I received, and, among thousands of spores of a single *Glomus* species, there was a single spore of *Gigaspora gigantea* (Nicol. & Gerd.) Gerd. & Trappe. This clearly demonstrates the need for very careful and detailed examination of pot cultures to ascertain purity.

Single spore isolates

The best way to overcome the problems of mixed inocula is to begin with single spore isolates and carefully to maintain their purity. Once established, a single spore isolate should be distributed to several laboratories so that if it becomes contaminated in one, it may be recovered from another. Such isolates are extremely valuable to the taxonomist, and I would certainly appreciate receiving material from them.

Conclusion

The system of taxonomy currently used in the endogonaceae is not perfect, but it is workable. The species concepts are clarifying and as more studies are made within the group, the problem of identification should diminish. Perhaps an effort should be made to achieve a consensus on taxonomic concepts to be used, and on more standardization of description terminology.

The beginnings of the "whole fungus" approach (Kendrick 1979), to the taxonomy of this group have been laid, with work on identification of endophytes in roots, chemotaxonomic and histological studies, and results of work on host reaction to different strains of the same species. Continuation of the philosophy should be encouraged, so that morphological studies can be reinforced. However, the taxonomy at species level should continue to be based on characteristics observable by competent light microscopy, thus enabling people without acces to sophisticated techniques to identify their endophytes.

I can do no better than to finish with a quotation from the Minutes of the AAAS Council Meeting, August 1983:

> "Therefore be it resolved that the American Association for the Advancement of Science recommends to the Government of the United States and its granting agencies that they recognize the fundamental importance of and need to support taxonomic research and services, faunal and floral surveys, and the production and publication of monographs and identification manuals."

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PROCEEDINGS OF TAXONOMY SESSION AT 6TH NACOM

Moderator: Christopher Walker

During the session on taxonomy of the Endogonaceae, several speakers made important contributions summarized as follows. These summaries were written from notes made by the moderator, and therefore any errors should be attributed to him rather than to the individuals concerned.

Jim Trappe, Corvallis, Oregon, U.S.A.

The erection of a taxon for organisms with particular characteristics is nothing more than the erection of the hypothesis that those organisms have characteristics genetically closer to each other than to entities not sharing such features. This, in common with all other hypotheses, is open to testing, and may be confirmed or rejected. It therefore should neither be surprising nor alarming when it becomes necessary to adjust the boundaries of a taxon. For example, boundaries for the species *Glomus fasciculatum* were set in 1974 by Gerdemann and Trappe (*Mycologia Memoir* No. 5). Later an organism was discovered that, although falling within these boundaries, was clearly different from the others that fitted the description. This was named *Glomus deserticola*. Other species will be separated from *G. fasciculatum* sensu lato as time passes.

Jack Gibson, Gainesville, Florida, U.S.A.

Studies on *Glaziella aurantiaca* show that the species is not endogonaceous, but is an ascomycete. The septa in the glebal hyphae possess Woronin bodies, and show other characteristics typical of ascomycetes. Studies of the large spores embedded in the gleba indicate that they are giant ascospores. More details are given in a poster presented at this conference and later publication will elucidate this further. The sporocarps are definitely not a juxtaposition of two different organisms.

Norman Schenck, Gainesville, Florida, U.S.A.

There are two new species of *Acaulospora* from Colombia, each of which has two distinctive spore forms. The fungi have typical "azygospores" each formed on a sporiferous saccule,

but also have chlamydospores that seem indistinguishable from those of *Glomus*. Descriptions of these species are currently in press, and in a poster at this conference. An interesting characteristic of one of these species is that the germ tube emerges directly through the subtending hypha, a manner generally thought to be typical of *Glomus spp*.

Paola Bonfante-Fasolo, Torino, Italy.

Investigations have been made into the ultrastructure of some endogonaceous spore walls. To begin with, *Glomus versiforme* spores were examined to see if the structure observed under the light microscope corresponded with that seen through the transmission electron microscope. The observations did indeed correspond well. The outer wall (designated a "unit wall" in the scheme of Walker 1983 (*Mycotaxon* 18:443-455)) is characterized by an ordered deposition of parallel fibrils. The inner wall, in contrast, consists of a laminated wall with as many as twelve layers that appear to be formed by fibrils laid down in arcs (Figure 1). These are really formed by the deposition of fibrils that are rotated through a small angle with respect to the one previously laid down. Examination of seven other members of the Endogonaceae, including *G. macrocarpum*, *G. clarum*, and *G. caledonicum*, shows that each has it own particular wall structure when examined by TEM, and these broadly reflect the structures observed by light microscopy. However, discrepancies do exist, especially where terminology is concerned. At the light microscope level, a layer is simply a separated sheet of wall material, whereas at the ultrastructural level, the term is reserved for a part of a wall in which the constituent fibrils show the same texture (Roland & Vian 1979, *Intern. Rev. Cytology* 61:129-166). The use of the term "layer" is probably best avoided, and the spores should be considered as being surrounded by a number of different distinctive walls.

Lynette Abbott, Nedlands, Australia.

Investigations into the morphology of the mycorrhizae caused by certain species of Endogonaceae show clearly that it is possible to use mycorrhizal characteristics to identify introduced endophytes. However, it is necessary to use known isolates with a particular host, under specified conditions. It is not possible simply to sample mycorrhizae at random and assign a fungal species to them. Characteristics that should be used include diameter of hyphae, number and configuration of entry points, position and direction of growth of hyphae in relation to cells and entry points, constrictions and swellings on hyphae, size, intensity, and shape of vesicles, morphology of external vesicles, and external hyphae. Characterization of mycorrhizae is of particular use for recognizing contamination in pot cultures and experimental material. There is a need to examine the effects of different hosts and conditions on the morphology of mycorrhizae. Such studies would be of great importance in pursuing the "whole fungus" approach to the taxonomy of endogonaceous fungi.

Barbara Mosse, Rothamsted, U.K.

It is important when studying the anatomy of colonization to examine sufficient material under different conditions to obtain a measure of the variation within and among species. Similarly, it is necessary to know the degree of variability to be found in spore wall layers both at the ultrastructural and light microscope level of observation.

Ian Hall, Mosgiel, New Zealand.

Immunofluorescent techniques can be used to examine both hyphae and spores of endogonaceous fungi. The work is in its early stages, but it does seem that there may be a place for such techniques in taxonomy. So far, success has been greatest at the generic level, and is limited by the need to obtain purified material in relatively large quantities. This highlights the need for the production of cultures under aseptic conditions.

Jim Gerdemann, Yachats, Oregon, U.S.A.

It is interesting to consider the phylogenetic relationships among species currently placed in the Endogonaceae. For example, could there be an evolutionary sequence from *Entrophospora* through *Acaulospora* to *Gigaspora*? Examination of the mode of spore formation suggests this, the sporiferous saccule gradually being lost as the sequence progresses.

Chris Walker, Edinburgh, U.K.

There are certainly two different groupings of *Gigaspora*, based on spore wall structure, germination characteristics, and auxiliary cell morphology. This could suggest an evolution from a sexual to an asexual mode, the former being represented by germ shield formers such as *G. calospora*, and the latter by those species that germinate directly through the spore wall, such as *G. gigantea* or *G. margarita*. There is a great need for ontogenetic and cytological studies of pure cultures of endogonaceous species.

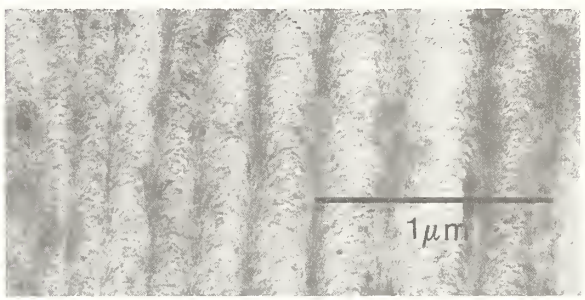


Figure 1: Transmission electron micrograph of the laminated spore wall of *Glomus versiforme*. The rotation of each layer of fibrils gives rise to the arched appearance seen here and the laminated appearance seen through the light microscope (see Bonfante-Fasolo & Vian (*Protoplasma* 120,51-60 (1984)).

ERICACEOUS AND ORCHIDACEOUS MYCORRHIZAE

PROGRESS, PROBLEMS AND PROSPECTS IN RESEARCH ON ERICACEOUS AND ORCHIDACEOUS MYCORRHIZAS

By

D. J. Read

Key words: Ericoid mycorrhiza, arbutoid mycorrhiza, monotropoid mycorrhiza, orchid mycorrhiza, organic nitrogen, carbon nutrition, achlorophylly

Introduction

Analysis of the mycorrhizal status of the Ericales and Orchidales reveals in each order a gradient of dependency from achlorophyllous species which obtain every resource by way of their fungal associate, to fully autotrophic plants which can grow normally with little or no infection. The parallelism of mycorrhizal function in the orders is closest in the achlorophyllous members the minute and structurally similar seeds of which lack the resources to provide the plant with an independent existence after germination. Most orchids and members of the Pyrolaceae and Monotropaceae, because they have seeds of this kind, are dependent upon symbiotic germination, and their distribution must in turn be influenced by the availability of appropriate fungi. In the Ericaceae, in contrast, most species produce larger seeds and can germinate without infection. However, their later success in some of the most stressed environments on earth appears to be associated with the development of the particularly specialised 'ericoid' mycorrhizal type (Read 1983).

Structure and function of mycorrhiza in the Ericales

A. Progress

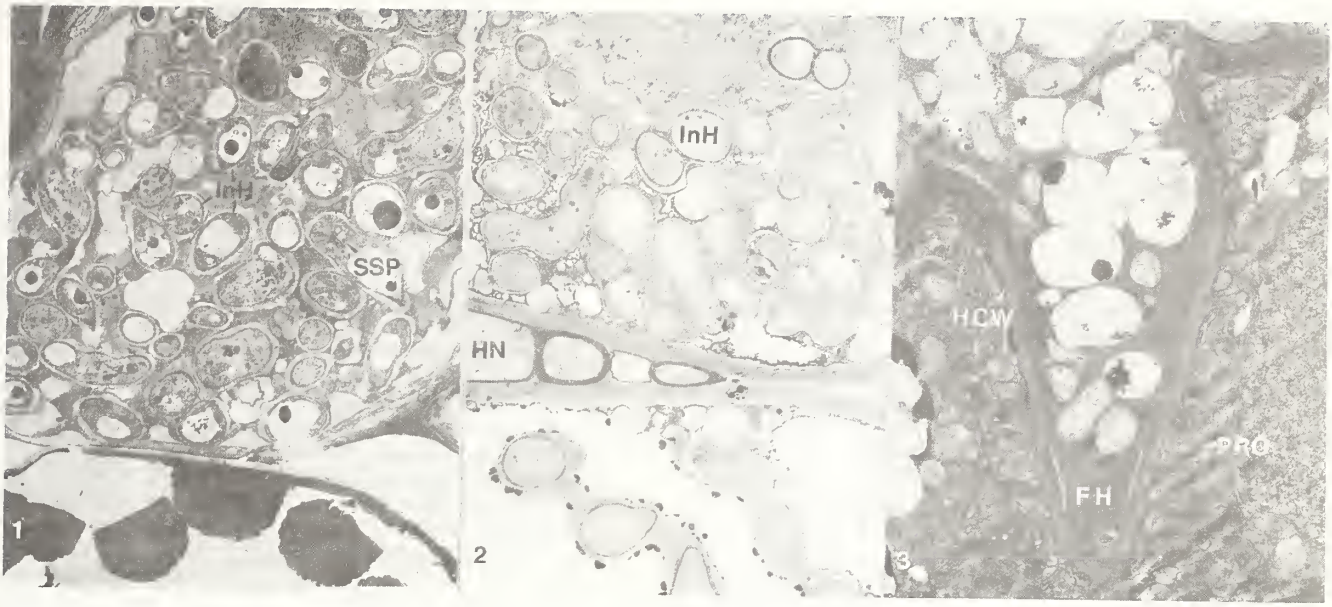
Three categories of mycorrhiza are recognized in the order. These are the 'ericoid' type with predominantly intra-cellular infection (Fig. 1) normally formed by the ascomycetous fungus Pezizella ericae, (= Hymenoscyphus ericae Kernen & Finocchio 1983), the arbutoid type (Fig. 2) which, having an internal phase together with Hartig net and sheath is intermediate between ericoid and ecto-types and can be formed by basidiomycetous or ascomycetous fungi (Fusconi & Bonfante-Fasolo 1984), and the 'monotropoid' mycorrhiza characterized by the formation of 'pegs' (Fig. 3) in the outer cortical cells in addition to sheath and Hartig net. Discomycete fruit bodies have been observed in association with Monotropa roots (Kernen & Finocchio, 1983). This type of mycorrhiza is restricted to achlorophyllous species and recent research shows that the peg, which arises when the host cell wall is caused to invaginate around an intruding fungal hypha, has the structural characteristics found in transfer cells (Duddridge & Read 1982, Robertson & Robertson 1982). Since pegs are produced most extensively during the period of shoot extension they may be involved in transfer of nutrients from fungus to host.

Ericoid mycorrhizas are found in those genera of the Ericaceae and Empetraceae like Calluna, Vaccinium, Erica and Empetrum which grow freely in acidic organic soils of high water and low oxygen status. Recent studies (Read & Bajwa in press, Spinner & Haselwandter, this volume) indicate that the fungus provides the plant with the capacity to utilize organic sources of nitrogen. The ericoid endophyte growing in pure culture uses amino-acids, peptides and proteins freely as nitrogen sources, and continues to do so when in association with a host plant. Yields of ericaceous plants are thus enhanced when they are grown on such nitrogen sources in the mycorrhizal condition (Fig. 4, Table 1). The endophyte provides a significant proteolytic capability which is lacking in the aseptically grown host plant and it is of great ecological interest that the pH optimum of this activity is around 4.0 (Fig. 5), which is the characteristic soil pH of heath environments. Analysis of such soils reveals that soluble amino acids are present in quantities as high as those of mineral nitrogen and that they occur mostly as neutral and basic forms. These amino acids are thought to be products of protein or peptide breakdown, since a similar spectrum of compounds is released by acid hydrolysis or peptidase enzymes (Jalal & Read in press). The results suggest that mycorrhizal plants may have access to a large pool of potentially hydrolysable organic N in these soils.

B. Problems

Progress in understanding the physiology of ericoid mycorrhizas has outstripped our knowledge of the chemistry of soil in which the plant grows. We need to know more of the chemical nature of the major organic nitrogen and phosphorus sources of soils before we can fully interpret the ecological significance of results obtained using pure compounds in the laboratory. We also know little of the carbon balance of ericaceous mycorrhizas. Does the fungus contribute carbon to the autotroph as it does in the Orchidaceae? In this context it is interesting to note that Oidiodendron griseum, recently reported to form ericoid mycorrhizas (Couture et al. 1983), has cellulolytic capabilities similar to those of many orchid endophytes. Histochemical analysis of the host-fungus interface after feeding labelled carbon to the respective partners in the symbiosis would help to resolve some of these questions. The nature of the relationship between Clavaria argillacea and ericaceous roots is still unclear despite evidence of reciprocal transfer of nutrients (Englander & Hull 1980) and the broader question of the capacity of basidiomycetes to form ericoid mycorrhizas has still to be resolved.

Further study is needed of the nature of the relationship between members of the Monotropaceae and Pyrolaceae and those ecto-mycorrhizal plants with which they are consistently found in nature. While Monotropa might be expected to benefit from connections with an autotroph, Pyrola is itself autotrophic. In many cases it is difficult to detect ^{14}C transfer to either plant from labelled autotrophs. Is this because the only truly



TEM of infected cortical cell of Rhododendron ponticum showing packed internal hyphae (InH) typical of the ericoid infection. A simple septal pore (SSP) confirms ascomycetous affinities of the endophyte. x4000.

TEM of infected cortical region of Pyrola rotundifolia showing arbutoid infection with both Hartig net (HN) and internal hyphae InH. x4000.

High power TEM of longitudinal section through the 'peg' of a cortical cell of Monotropa, showing encapsulated fungal hypha (FH), invaginated host cell wall (HCW), and proliferation of material from the peg wall (PRO). x13,965. (Photos of J A Duddridge)

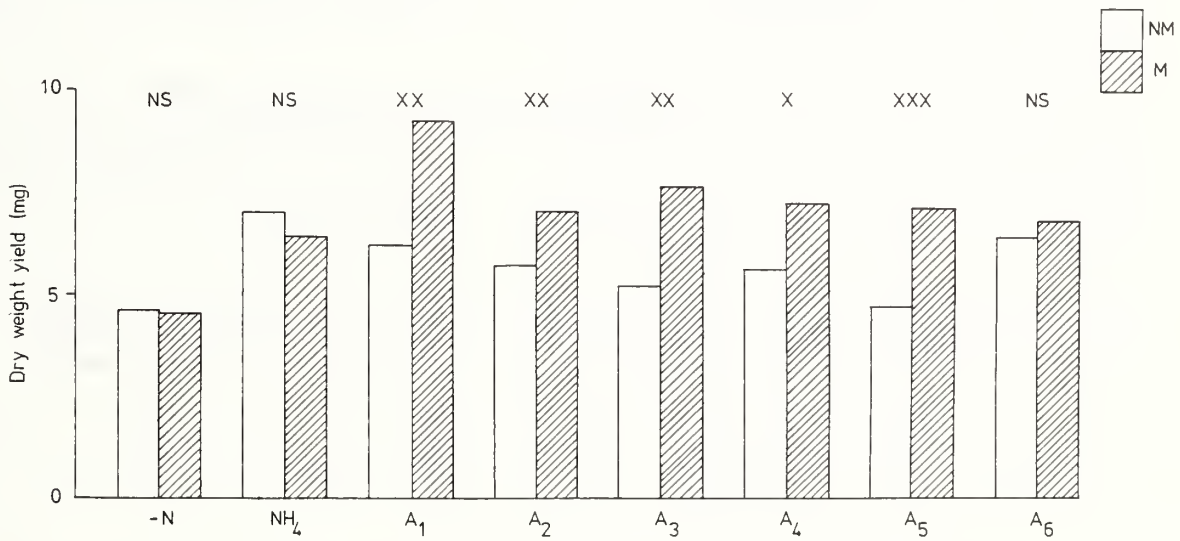


Fig. 4. Yield of mycorrhizal (M) and non-mycorrhizal (NM) plants of Vaccinium corymbosum after growth for 60 days in solution lacking nitrogen (-N), with ammonium as sole N source, or with peptides made up of increasing numbers of alanine units from the amino acid level to hexa-alanine (A6). *** P = < 0.001, ** P = < 0.05, * P = < 0.01.

dependent stage is that involving symbiotic germination? Failure to detect transfer in Monotropa could be attributable to the fact that the expansion of the flowering scape is sustained by reserves accumulated in below ground organs the previous year. The 'transfer cells' would then be involved simply in re-mobilisation of these reserves. An alternative explanation is that most of the carbon requirements of monotropoid plants come, as they appear to do in achlorophyllous orchids, from litter breakdown. It is a testament to the remarkable observational and manipulative skills of Kamienski that despite the tremendous increase of technological capability we have advanced little in our understanding of the basic biology of the 'monotropoid' association since his description of this mycorrhizal structure in 1881.

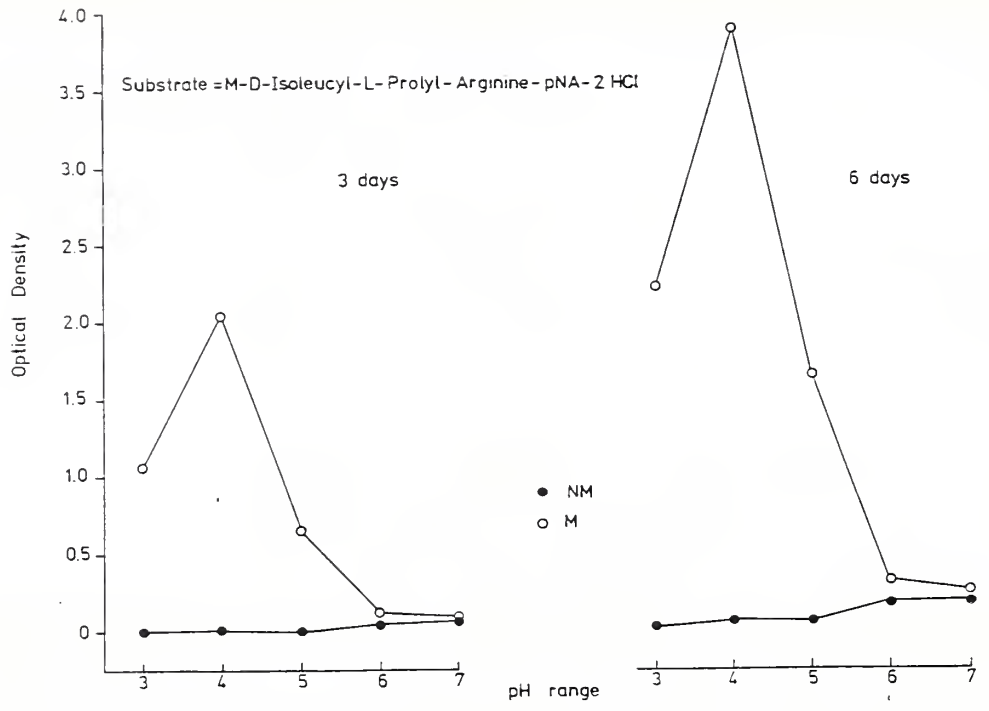


Fig. 5. Proteolytic activity of mycorrhizal (M) and non-mycorrhizal (NM) seedlings of Vaccinium corymbosum. Seedlings were grown on pH adjusted mineral nutrient solution with chromogenic substrate, and enzyme activity was assayed spectrophotometrically after 3 or 6 days of incubation.

Table 2. Effect of mycorrhizal infection on fruit yield and gross financial return in commercial blueberry crops of New Zealand. N.Z. Ministry of Agriculture data.

Year	Mycorrhiza status	Fruit Yield g/plant	Gross return $/ha assuming $5 kg for fruit
1979-80	Non-inoculated	523	6213
	Inoculated	704	8363 (+ $2,150)
1980-81	Non-inoculated	723	9034
	Inoculated	911	11384 (+ $2,350)

C. Prospects

As evidence of the nutritional significance of mycorrhizas accumulates, the prospect is of a greater awareness of their role in ecosystems. Ecologists have long recognized that distinctive shoot phenologies are associated with particular climatic circumstances but have been slow to observe that roots in particular soil circumstances have distinctive mycorrhizal associations. It is now clear that in ericoid mycorrhizas we have not only a structural but a functional specialization which provides the potential for access to organic nitrogen resources otherwise unavailable to plants. Such a feature is likely to be of central importance to the nutrient dynamics of the ecosystem.

The importance of ericoid mycorrhizas is now being recognized on a commercial scale in New Zealand (Table 2) where inoculation has produced significant increases of yield in blueberry crops. Screening of isolates for maximum effectiveness will follow and there are clearly prospects for wider use of inoculation in horticulture as the direct effects of improved nutrient status and the indirect benefits of heavy metal and pathogen exclusion are recognized by growers.

Structure and function of mycorrhizas in Orchidales

A. Progress

The fungi of orchid mycorrhizas appear to be mostly basidiomycetes, the hyphae of which produce characteristic coils in the fleshy cortical tissues of the roots. Warcup (1981) taxonomically identified a number of basidiomycete fungi isolated from orchids and demonstrated an element of specificity in some of the associations. Acknowledgement of the importance of specificity forms the basis of recent research by Clemence, who, developing the work of Warcup, has shown that of a number of 'difficult' species can be induced to germinate when inoculated with the appropriate fungus. Progress in obtaining symbiotic germination has gone along with advances in understanding of the requirements for asymbiotic germination, which has recently been achieved in the achlorophyllous species Galeola septentrionalis by Nakamura (1982).

The mycorrhizal fungi are known to produce cellulolytic, pectinolytic and even ligninolytic enzymes and the carbon compounds arising from this lysis have been shown to be transferred to developing orchids. These results imply that in natural situations the ultimate sources of carbon, for young plants at least, are organic residues in soil, a situation which contrasts with that believed to occur in the Monotropaceae, where nearby autrotrophs are thought to provide carbon.

While much has been learned concerning the role of mycorrhizas in early development of the orchid plant, we know little of its function in later stages. Recent work by Alexander and Hadley (1984) has demonstrated the importance of the external phase of the association in young plants. They showed that if the fungal mycelium attached to Goodyera repens roots is killed by the fungicide thiabendazole (TBZ), growth of the plants is significantly reduced, as is their phosphorus content (Table 3). This suggests that orchids may be dependent on the external mycelium for nutrient absorption in the same way as are many VA and ecto-mycorrhizal plants.

B. Problems

The relatively massive roots of many orchid species seem to be singularly ill-equipped for soil exploitation and it might be expected that an effective external mycelial system would be essential for survival of mature plants in these circumstances. Many orchids grow in species rich vegetation on water and nutrient stressed soils, yet we still know virtually nothing about their water or mineral nutrient relations, even less about the possible role of mycorrhizas, in the post-establishment phase of their lives. Experiments on the water relations, and on the nitrogen and phosphorus nutrition of mycorrhizal and non-mycorrhizal orchid plants are urgently needed.

C. Prospects

Changes of land-use and increasing collection of blooms by man pose a threat to many orchid species. The progress currently being made an understanding the requirements for symbiotic and asymbiotic germination may enable us effectively to conserve endangered species. Seedlings raised symbiotically in both Australia and at Kew have been re-introduced to nature reserves. Improved knowledge of the factors involved in orchid germination could lead to routine culture even of achlorophyllous species, and this in turn, if it provides mature plants, would enable us to investigate the carbon and mineral nutrition of orchids under more realistic circumstances than has hitherto been possible.

Table 3. The P content, mean relative growth rate (RGR) and R:S ratio of Goodyera repens: (i) uninfected plantlets; (ii) plantlets inoculated at the start of the experiment; (iii) fungicide treated (1 μg ml^{-1} TBZ) uninfected plantlets and (iv) mycorrhizal plantlets infected from seed. From Alexander and Hadley New Phytol. in press.

Treatment	RGR 0-6 wk (mg mg^{-1} wk^{-1} x 10^{-2})	RGR 0-22 wk (mg mg^{-1} wk^{-1} x 10^{-2})	Wet wt at 22 wk (mg)	P content (μg/g d.wt.)	R:S ratio (dry wt)
Uninfected (control)	7.08^{a}	3.91^{a}	59	$2,750^{a}$	1.42^{a}
Inoculated at start of experiment	4.84^{a}	2.12^{b}	44	$4,737^{b}$	1.24^{ab}
Uninfected, fungicide treated	5.18^{a}	3.02^{ab}	52	$2,252^{a}$	1.75^{a}
Mycorrhizal	27.86^{b}	12.95^{c}	409	$4,635^{b}$	0.85^{b}

n = 13

Similar technology should be applicable to germination of achlorophyllous ericaceous plants. The prospect of being able to investigate the nutrient relations of Monotropa plants developing in association with ecto-mycorrhizal trees in a root chamber is exciting indeed.

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POSTER SUMMARIES

PRACTICAL APPLICATIONS

EFFECT OF DIFFERENT MYCORRHIZAL FUNGI ON PINUS RADIATA SEEDLING GROWTH

By

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New Zealand

Keywords - Rhizopogon, Laccaria, Endogone, Hebeloma, Thelephora, Tuber

Introduction

Pinus radiata, exotic in New Zealand is associated with a limited number of mycorrhizal fungi compared with its indigenous state (Chu-Chou, 1979; Trappe, 1962). This study examined the efficiency of the commonest mycorrhizal fungi of radiata pine in nurseries, on seedling growth and nutrient uptake.

Materials and Methods

Pot trials were used to compare the efficiency of different mycorrhizal fungi on seedling growth either under controlled environment in a growth cabinet or in a glasshouse.

Spore suspension, prepared from fresh sporocarps of each fungus (Rhizopogon luteolus, R. rubescens, Laccaria laccata, Endogone flammicorona, Hebeloma crustuliniforme, Thelephora terrestris, and Tuber sp.), was used as inoculum. Seedlings were inoculated twice, at 4 and 8 weeks old.

Height of seedlings was measured monthly. Nitrogen and phosphorus content of shoots were analysed at 6 and 11 months and at the same time the root systems of some of the seedlings were examined for the purity of their mycorrhizas.

Results and Discussion

The results show that R. rubescens was the most efficient mycorrhizal fungus for seedling height growth (Fig. 1) as well as nitrogen and phosphorus uptake (Fig. 2 and 3). Hebeloma crustuliniforme and T. terrestris were not

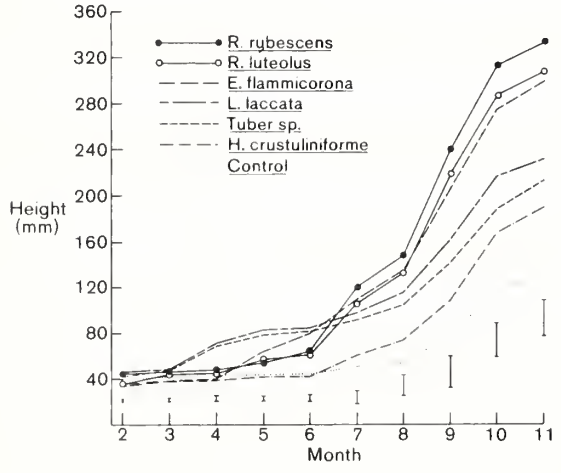

Fig. 1. Height growth of radiata pine seedlings inoculated with different mycorrhizal fungi (bars below the graph show LSD at 5% level).

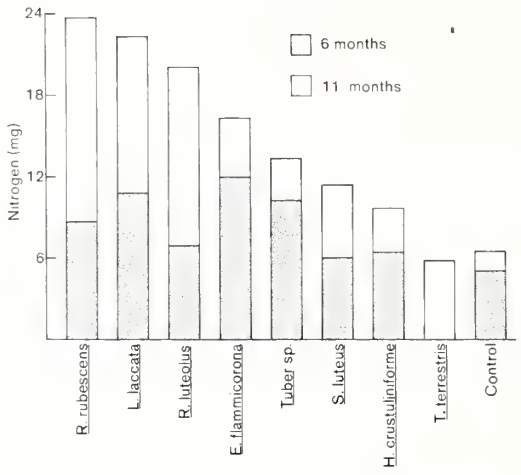

Fig. 2. Nitrogen content of radiata pine seedlings inoculated with different mycorrhizal fungi at 6 and 11 months.

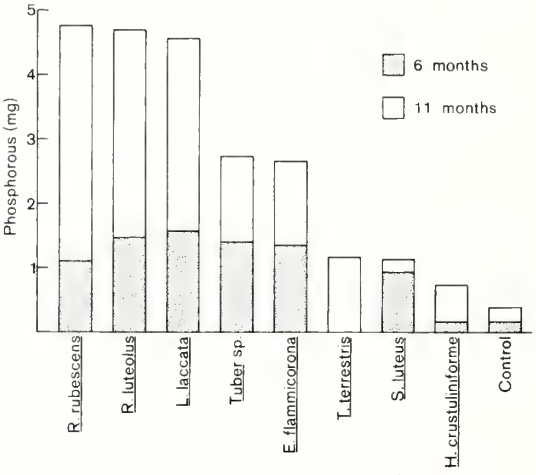

Fig. 3 Phosphorus content of radiata pine seedlings inoculated with different mycorrhizal fungi at 6 and 11 months.

effective in promoting seedling growth and nutrient uptake. Laccaria laccata and Tuber sp. stimulated the early growth of seedlings (between 3 and 6 months) but the effect gradually declined as the seedlings became older. This could be due to differences in the time of infection of these fungi or other unknown causes.

Mycorrhizas formed by Rhizopogon spp. persisted in a pure state throughout the experiment, but seedlings inoculated with other fungi were usually contaminated with Rhizopogon spp. by the end of the experiment (11 months). There seem to be differences in the durability of host association among these mycorrhizal fungi studied.

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NURSERY INOCULATION OF DOUGLAS-FIR SEEDLINGS WITH COMMERCIALLY PRODUCED ECTOMYCORRHIZAL INOCULUM

By

Ling-Ling Hung and Randy Molina

Keywords--Laccaria laccata, containerized seedlings.

Introduction

Laccaria laccata, a common ectomycorrhizal fungus forms excellent mycorrhizae with Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) containerized seedlings under high fertility (Molina & Chamard, 1983). Preliminary tests indicated that L. laccata mycelial inoculum produced by Sylvan Spawn Laboratory was a successful inoculum with Douglas-fir seedlings in a greenhouse. Our study was designed to determine (1) the effectiveness of Sylvan Spawn inoculum to form mycorrhizae with Douglas-fir containerized seedlings in commercial nurseries, (2) the optimum inoculation rate in a research greenhouse and in commercial nurseries, and (3) the effectiveness of the inoculum after storage up to 1 year.

Materials and Methods

Experiment 1 was a completely randomized design with eight inoculation rates of L. laccata inoculum to potting substrate (v/v) of 0, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, and 1:256. Experiment 2 was a completely randomized block design in each of two cooperating container nurseries with five inoculation rates (0, 1:4, 1:16, 1:64, and 1:128) in each of four blocks. Experiment 3 inoculation treatments were set up in a walk-in growth chamber. Inoculum was either used immediately after receipt (fresh) or stored. The inoculum was stored either in a cold room (ca. 2°C) or at room temperature (ca. 21°C) for different periods up to 1 year.

Results and Discussion

Experiment 1. Inoculated seedlings formed L. laccata mycorrhizae at the first harvest (8^{th} week). At the end of the experiment, primordia and fruiting bodies of L. laccata developed on all inoculation treatments. In general, inoculated seedlings were taller and had heavier shoot and total weights and more feeder roots, and at least 65% were colonized by L. laccata. Within the inoculation treatments, no significant differences were detected for dry weights, total number of laterals, and percentage of mycorrhizae. Seedlings inoculated at the rate of 1:128 had 79% of their feeder roots colonized by Laccaria and seedling growth equaled that following other inoculation.

Experiment 2. Seedlings at different nurseries responded differently to inoculation rate. In general, the best treatment at International Paper Company was at 1:64 application rate. Inoculated seedlings had 73.8% to 80.2% of their feeder roots colonized by L. laccata. At Champion Nursery, L. laccata inoculation significantly improved percentage of Laccaria and total mycorrhizae over noninoculated controls. Percentage of feeder roots with Laccaria generally increased with increased inoculation rate, as did seedling root and total weights.

Experiment 3. Seedlings inoculated with fresh inoculum formed significantly more mycorrhizae than the means of those receiving stored inoculum (86.42% vs. 29.12%). Mycorrhiza-forming ability of L. laccata inoculum decreased with increasing storage time beyond 2 months. Inoculum stored in a cold room remained viable and effective longer than that stored at room temperature (Fig. 1). Within a particular storage period, differences among different inoculation rates increased with increased storage time. This trend was much striking when seedlings inoculated at the highest and lowest rates were compared.

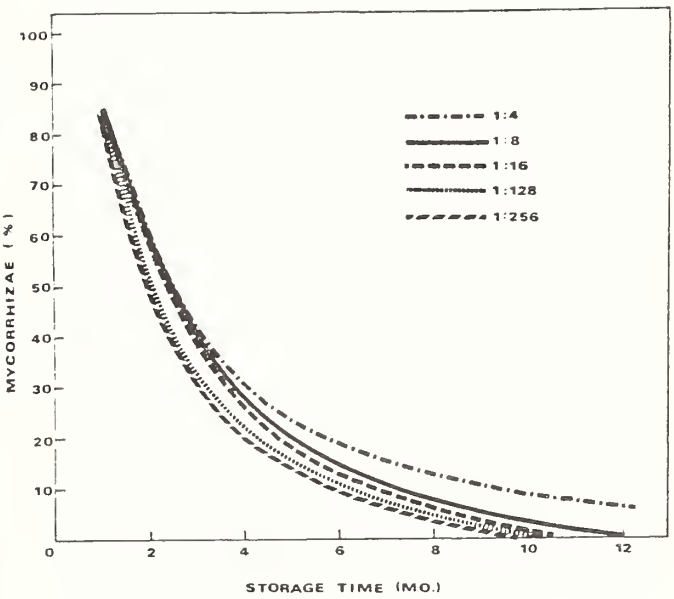

Figure 1. Regression curves of Laccaria laccata mycorrhiza formation with inoculum stored for different periods and applied at different rates.

Conclusions

(1) Sylvan Spawn inoculum of L. laccata was effective under commercial nursery operation.
(2) The inoculum was effective at rates as low as 1:128.
(3) Inoculum can be stored at 2°C or room temperature (ca. 21°C) for 2 months and remain effective (form at least 40% mycorrhizae).

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ECTOMYCORRHIZA INOCULATION OF DOUGLAS-FIR PLUG+1 SEEDLINGS WITH COMMERCIALLY PRODUCED INOCULUM

By

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Keywords--Hebeloma crustuliniforme, Laccaria laccata, Pisolithus tinctorius, Thelephora, Rhizopogon, transplanting.

Introduction

"Plug+1" seedlings, grown initially in containers and then transplanted to nursery beds, are one of the newer seedling types used for reforestation. They survive and grow well on typical Pacific Northwest sites (Hahn, 1984). Inoculation of plug+1 seedlings with mycorrhizal inoculum during the container growing phase may reduce the shock of transplanting seedlings to nurerry beds. This study was set up with commercially produced inocula of Hebeloma crustuliniforme (Bull.:St. Am.) Quel. S-166 (Hc), Laccaria laccata (Scop.:Fr.) Berk & Br. S-238A (Ll), and Pisolithus tinctorius (Pers.) Coker & Couch S-216 (Pt) to (1) determine the effectiveness of inoculum to form mycorrhizae with Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) plug+1 seedlings, (2) detect differences among fungal species in effects on seedling growth and mycorrhiza development, and (3) determine whether the inoculated fungus persists throughout growing season after transplanting.

Materials and Methods

The study used a randomized block design with four fungal treatments and four blocks. Douglas-fir seedlings were inoculated with Hc, Ll, or Pt, vs. noninoculated control and grown in "Ray Leach" containers (65 ml capacity each) filled with 1:1 (v/v) vermiculite and peatmoss. Mycelial inoculum was provided by Sylvan Spawn Laboratories, Worthington, Pennsylvania. The study was installed at International Paper Company, Western Research Center at Labenon, Oregon. Seedlings were grown in a greenhouse for one growing season (May 1982) and then transplanted (October 1982) into nursery beds for another season.

Results and Discussion

At the end of their growing phase in containers, seedlings appeared healthy and had set buds, with mean shoot height of 14.6 cm and stem diameter of 1.94 mm. Significant differences between inoculation treatments occurred only on number of feeder roots and percentage of mycorrhizae. Ll- and Hc-inoculated seedlings formed abundant mycorrhizae with 83.0% and 90.2%, respectively, of their feeder roots colonized by inoculated fungus. Pt-inoculated seedlings formed only 4.0% mycorrhizae. Ll- and Hc-inoculated seedlings had significantly more mycorrhizal and total feeder roots, and less nonmycorrhizal short roots than Pt-inoculated or noninoculated seedlings (Table 1).

Table 1. Mean feeder roots and mycorrhiza formation of Douglas-fir seedlings inoculated with different fungal inocula, grown in a greenhouse, for 4.5 months.

Treatment	Number of Feeder Root Myc	Nonmyc	Total	Mycorrhizae (%)
Control	0b*	175a	176b	0.0c
Hc	264a	28b	292a	90.2a
Ll	234a	43b	277a	83.0b
Pt	9b	170a	186b	4.0c

* Means in the same column not sharing a common letter differ significantly by Scheffe's test at $P \leq 0.05$.
Myc - mycorrhizal feeder root.
Nonmyc - nonmycorrhizal feeder root.

Foliage nutrient analysis indicated that all inoculated seedlings had significantly higher Zn levels and all but Ll-inoculated seedlings had significantly lower Mn levels as compared to noninoculated controls (Table 2). After transplanting to nursery beds and grown there for 17 months, all seedlings were 80% mycorrhizal. Hc persisted as a mycorrhizal dominant on seedlings previously inoculated with this fungus. Ll-inoculated seedlings had 40% Ll mycorrhizae and another 40% formed with native fungi (Rhizopogon(Rh) and Thelephora(Th) spp.). All mycorrhizae of the Pt-inoculated and noninoculated seedlings were formed with the native fungi (Table 3).

Table 2. Mean foliage nutrient levels of Douglas-fir container seedlings grown in a greenhouse for 4.5 months.

Treatment	Mn (ppm)	Zn (ppm)
Control	299a*	54b
Hc	205b	87a
Ll	231ab	97a
Pt	200b	96a

* Means in the same column not sharing a common letter differ significantly by Tukey's test at $P \leq 0.05$.

Table 3. Mycorrhiza formation (percent) of Douglas-fir plug+1 seedlings after transplanting.

Types Treatment	Hc	Pt	Ll	Rh	Th
Control	--	--	--	20	60
Hc	70	--	--	10	0
Ll	--	--	40	20	20
Pt	--	0	--	20	60

Reference Cited

Hahn, P. F. 1984. Plug + 1 seedling production. In Forest nursery manual: Production of bareroot seedlings. Edited by M. L. Duryea and T. D. Landis. Martinus Nijhoff/Dr. W. Junk Publishers. The Hague/Boston/Lancaster. p. 165-191.

BASIDIOSPORES OF RHIZOPOGON VINICOLOR AND RHIZOPOGON COLOSSUS AS ECTOMYCORRHIZAL INOCULUM

by

Michael A. Castellano

Keywords--Rhizopogon, inoculation, basidiospores

EXPERIMENT 1

Five spore rates (0, 10^3, 10^4, 10^5, 10^6 spores/seedling) of R. vinicolor and R. colossus were inoculated onto container-grown Douglas-fir seedlings and grown under three fertility regimes: HIGH = soluble 20-19-18 NPK fertilizer (biweekly schedule: weeks 4-6, 7 mg/seedling; weeks 8-20, 18 mg/seedling); LOW = 1/2 the HIGH rate; Slow-release (18-6-12 NPK) fertilizer was applied to substrate at 7.1 g/liter of substrate.

RESULTS

Both fungi formed abundant (> 54%) mycorrhizae under the soluble fertilizer regimes compared to the slow-release fertilizer which reduced percent mycorrhizae for both fungi. Stem height was significantly increased under LOW fertility with all R. colossus spore rates and the three lowest spore rates of R. vinicolor (Table 1).

EXPERIMENT 2

Four different conifers (sugar pine, white fir, western hemlock, and Douglas-fir) were inoculated with three spore rates (CONTROL = 0, LOW = 10^5, MEDIUM = 10^6, and HIGH = 10^7 spores per ft^2) of six hypogeous ectomycorrhizal fungi (R. vinicolor, R. colossus, R. ochraceo-rubens, Gauteria monticola, Hysterangium separabile, and Hymenogaster parksii) and grown for two years at the International Paper Co. bareroot nursery.

RESULTS

For seedlings inoculated with R. vinicolor, the HIGH rate produced the most mycorrhizae on the greatest number of seedlings. For seedlings inoculated with R. colossus, the HIGH spore rate produced the most mycorrhizae on the greatest number of seedlings (Table 2). No significant differences in stem height or diameter occured.

EXPERIMENT 3

Bareroot 2-0 Douglas-fir seedlings grown in a nusery bed (not inoculated or inoculated with R. vinicolor spores) were outplanted on a routine reforestation site in southern Oregon which was formerly occupied by Alnus rubra.

RESULTS

After two years, inoculated seedlings had significantly increased survival, stem height, stem diameter, and seedling biomass (PVI) compared to noninoculated seedlings (Table 3).

DISCUSSION

Lack of information on which fungus species can be effectively used for spore inoculation presently limits development of this technology. Of particular interest in the Pacific Northwest are species of Rhizopogon. Douglas-fir seedlings mycorrhizal with R. vinicolor have been shown to tolerant drought better than either nonmycorrhizal seedlings or those mycorrhizal with other fungi. Rhizopogon vinicolor ectomycorrhizae are characterized by rhizomorphs which have been shown to function in water and nutrient transport. Rhizopogon vinicolor has also been shown to inhibit growth of root pathogens in vitro. Although problems remain in developing spore inoculation technology, use of spore inoculum remains an alternative to pure culture mycelium.

TABLE 1. Growth and mycorrhiza development of container-grown Douglas-fir seedlings inoculated with Rhizopogon vinicolor and R. colossus spores.

SPORE RATE	RHIZOPOGON VINICOLOR HEIGHT (cm)	RHIZOPOGON VINICOLOR MYCORRHIZAE (%)	RHIZOPOGON COLOSSUS HEIGHT (cm)	RHIZOPOGON COLOSSUS MYCORRHIZAE (%)
	LOW Fertility Regime			
10^3	12.84a	95.3a	13.92a	54.6a
10^4	12.97a	97.2a	14.54a	67.9ab
10^5	13.32a	95.7a	14.59a	72.9b
10^6	12.66ab	89.1a	14.68a	75.1b
0	12.15b	0.0b	12.15b	0.0c
	HIGH Fertility Regime			
10^3	14.63a	88.6a	21.27a	76.3a
10^4	14.43a	97.6b	20.36a	80.1
10^5	16.91b	99.2b	20.02a	95.1b
10^6	17.31b	98.9b	20.08a	98.2b
0	20.03c	0.0c	20.03a	98.2b
	OSMOCOTE Fertility Regime			
10^3	29.88a	9.6a	29.04a	3.7a
10^4	30.93a	7.5a	29.59a	7.8a
10^5	31.54a	5.9a	29.26a	5.6a
10^6	31.98a	1.4a	31.17a	0.7a
0	31.25a	0.0b	31.25a	0.0b

Means within fertility regimes not sharing a common letter differ significantly by Tukey's test (P=.05).

TABLE 2. Two-year-old Douglas-fir inoculated with R. vinicolor or R. colossus basidiospores.

FUNGUS	SPORE RATE	SEEDLINGS WITH MYCORRHIZAE (%)	MYCORRHIZAE PER SEEDLING (%)
R.vinicolor	LOW	0.0a	0.0a
	MEDIUM	0.0a	0.0a
	HIGH	100.0b	44.4b
R. colossus	LOW	80.0b	25.6b
	MEDIUM	8.3a	2.0a
	HIGH	100.0b	48.6b

Treatment means not sharing a common letter differ significantly by Tukey's test (P=.05).

TABLE 3. Second year Douglas-fir seedling survival and growth.

TREATMENT	SEEDLING HEIGHT (cm)	STEM CALIPER (mm)	SEEDLING SURVIVAL (%)	PVI 2/ (cm^3)
Inoculated	61.74a	14.2a	93.6a	46.61a
Control	58.57b	13.4b	82.9b	34.87b

Treatment means not sharing a common letter differ significantly by Tukey's test (P=.05).

2/ PVI (Plot Volume Index) = (root collar diameter)^2 x stem height x number of surviving seedlings/plot.

DEVELOPMENT OF AN ECTOMYCORRHIZAL INOCULATION PROCEDURE FOR MICROPROPAGATED *Eucalyptus* PLANTLETS

By

N. Malajczuk and V.J. Hartney

Keywords--*Eucalyptus camaldulensis*, micropropagation, *Pisolithus*, *Scleroderma*

Introduction

The use of micropropagation is becoming increasingly important in the production of cloned material of desired characteristics in many aspects of forest regeneration programs (Bonga and Durzan, 1982). Similarly, the inoculation of tree seedlings with suitable ectomycorrhizal fungi for improving outplanting survival and growth stimulations is now well established practice in forestry (Trappe 1977; Marx 1980). The obvious advantages offered by combining these two forestry practices for maximizing gains from outplanted tree seedlings has yet to be demonstrated.

This preliminary study sets out to develop a simple mycorrhizal inoculation procedure for the *in vitro* production of ectomycorrhizal *Eucalyptus* seedlings for subsequent outplanting in the field.

Methods and Materials

A clonal line of *E. camaldulensis* Dehnh (CML 48) was produced according to the procedure described by Hartney and Barker (1980). All plantlets were transferred to root initiating media prior to use.

A range of ectomycorrhizal symbionts common to forest areas in south western Australia were isolated from sporocarps onto Melin Norkran's media (MN) and maintained in culture at 25°C.

Growth of both *E. camaldulensis* plantlets and fungi were investigated on 1. the root culture media, and 2. on MN agar after transplant of plantlets following root initiation onto this media. This agar was selected because it is routinely used for growing ectomycorrhizal cultures.

After 4 weeks fungal growth on both the media, plantlets were transplanted into Super cell containers[1] filled with sterile white sand and peat (4:1 mix) and fertilized with 0.5g/tube of slow release osmocote (18-2.6-10, N-P-K). Transplant survival and mycorrhizal development was recorded after 4 and 8 weeks growth.

Results and Discussion

Both root and shoot growth of *E. camaldulensis* seedlings were generally improved following inoculation with the various ectomycorrhizal fungi on both plantlet and fungal growth media. *Pisolithus* and *Scleroderma* isolates were the most effective in stimulating these growth responses and mycorrhizal formation was observed on roots on or above the agar surface (Table 1). Transplant survival of plantlets was significantly improved following inoculation with a *P. tinctorius* isolate *S. paradoxum* and a *Hysterangium* sp. Mycorrhizal formation was observed on plantlets inoculated with both *Pisolithus* isolates at 4 weeks and all, except the *Hysterangium* isolate, had extensive mycorrhizal development at 8 weeks (Table 2).

These results indicate that the integration of the mycorrhizal inoculum of selected fungal isolates with micropropagated plantlets is possible at the root initiation stage on root culture media, and that both growth and transplant survival can be significantly increased.

This method has the advantage over existing mycorrhizal inoculation techniques (Marx 1980) in that the fungus is pre-located on the root surface, thus avoiding the need for inoculum to survive in soil.

Table 1. Growth* of eucalypt seedlings and ectomycorrhizal fungi on agar media+ after 1 month incubation

Fungus sp.	Root Growth RC	Root Growth MN	Shoot Growth RC	Shoot Growth MN
Pisolithus tinctorius	++(M)	+++(M)	++	++
Pisolithus microcarpus	+++	+++	+++	++
Scleroderma paradoxum	++(M)	++(M)	+	++
Scleroderma verrucosum	+(M)	+++(M)	+	++
Hysterangium sp.	+++	+++	-	-
Hydnangium carneum	+	++	+	+
Hymenogastor albellus	+	+	-	-
Sclerogaster sp.	+	+	-	-
Laccaria laccata	+++(M)	+++	+	+
Uninoculated	+	+	-	-

*/Shoot and root growth rated at: +++ = >10mm; ++ = >0.5-10mm; + 0.1-0.5; - = no growth. (M) indicates presence of ectomycorrhiza formation in tissue culture container.

+/RC = root culture medium; MN - Melin Norkran medium.

Table 2. Percent transplant survival+ and mycorrhizal root formation* of *E. camaldulensis* plantlets.

Fungus sp.	Percent transplant survival	Mycorrhizal form. 4 weeks	Mycorrhizal form. 8 weeks
Pisolithus tinctorius			
Isolate 1	65a	1	3
2	80b	1	2
Scleroderma paradoxum	85b	0	2
Hysterangium sp.	71b	0	0
Laccaria laccata	63a	0	3
Control	50a	0	0

+/Means within a column not sharing a common letter differ significantly ($p<0.05$) by Duncan t test.

*/Mycorrhizal formation rated as 0, no infection 5, 80-100% infection of feeder roots.

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[1]Ray Leach "Cone-tainer Nursery" 1787 N. Pine St., Canby, OR., U.S.A.

MYCORRHIZAL GROWTH ENHANCEMENT IN SITKA SPRUCE SEEDLINGS DIFFERS IN NONSTERILE COMPARED TO STERILIZED SOIL

By

Christopher Walker

Keywords -- *Picea sitchensis, Laccaria proxima, Thelephora terrestris*, spruce mycorrhizae

Introduction

A project is underway to see if manipulation of ectomycorrhizae can increase growth and aid establishment of Sitka spruce (*Picea sitchensis* (Bong.) Carr.), the major tree used on British forestry sites, thus perhaps reducing the need for fertilizer application.

Evidence exists that mycorrhizal fungi from high-nutrient nursery soils may be less effective in forest conditions than those isolated from the field (Holden et al. 1983) and that some fungi are more capable of sustaining the symbiosis with young trees, whereas others normally form mycorrhizae only with older plants (Mason et al. 1983). Hence it is important that fungi are selected that can form vigorous mycorrhizae in nursery conditions, and can also survive after the trees have been transplanted to the forest.

Two isolates of mycorrhizal fungi were tested with Sitka spruce in a nursery soil. The experiment was split between untreated soil, and soil sterilized by irradiation.

Materials and Methods

Soil was dug from the Forestry Commission's nursery at Bush, Midlothian, Scotland, and mixed with coarse grit (1:1 v/v) to aid drainage. The mixture was then packed, double-wrapped, into polyethylene bags, half of which were irradiated with 5.4 Mrad gamma-radiation.

Square black plastic pots (13 x 13 cm) of approximately 1 litre volume were filled with the prepared potting mixture and watered thoroughly twice a day for a week to wash out any toxins that may have been induced by the irradiation. Sitka spruce seeds, of Queen Charlotte Island provenance, were soaked, chilled (Low 1975), and sown, twelve to a pot, in a greenhouse with minimum 15°C night temperature and a maximum of about 25°C.

Inocula of immobilized pellets of *Laccaria proxima* (Boud.) Pat. and *Thelephora terrestris* Ehr. ex Fr. in a liquid medium were prepared by N. Plummer, University of Surrey. The mycelium was filtered onto a fine sieve (45 µm openings), thoroughly rinsed in tapwater, and re-suspended in fresh tapwater. Half of each suspension was filtered through Whatman No. 3 filter paper and then through a 2 µm Millipore filter to provide a mycelium-free control. There were thus five treatments, *L. proxima* mycelial pellets, *T. terrestris* mycelial pellets, filtrate from each fungus, and tapwater control. Each plant received 10 ml of the appropriate treatment, injected into the root zone. There were ten replications of each treatment in a completely randomized design.

Destructive analysis was carried out 165 days after inoculation. Height growth was measured, and the data were subjected to analysis of variance, differences being located by use of Student's t-test between means.

Results and conclusions

There were no significant treatment-sterility interactions, and significant differences ($p<0.05$) occurred among treatments (Table 1).

Table 1: Mean SS seedling height 165 days after inoculation with mycorrhizal fungi.

Treatment	Mean height (mm)	
	S*	NS*
Laccaria pellets	133.50a	85.20c
Thelephora pellets	128.75a	73.90c
Laccaria filtrate	112.95b	73.60c
Thelephora filtrate	105.20b	70.20c
Tapwater control	97.95b	73.25c

*S = sterilized soil; NS = non-sterilized soil. Values followed by the same letter are not significantly different ($p<0.05$).

Sterilizing soil in itself stimulated growth, and addition of filtrate from the fungi did not increase growth significantly, though the filtrate control trees were slightly larger. *Laccaria proxima* and *Thelephora terrestris* both enhanced growth, but not significantly differently from each other on the sterilized soil. None of the other treatments enhanced growth compared with the non-sterile tapwater control. Thus, with these two isolates, only in sterilized soil was the introduction of mycorrhizae beneficial to height growth.

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MYCORRHIZA DIFFERENCES AMONG SITKA SPRUCE CLONES

By

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Keywords -- *Picea sitchensis*, Sitka spruce, cuttings, clonal differences, ectomycorrhizae

Introduction

The Forestry Commission in Great Britain is investigating the use of cuttings of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) in commercial forestry, and techniques have been developed for rooting large numbers of them under mist in polyethylene greenhouses. Cuttings are an ideal medium for studying mycorrhizal effects, since they have no roots at the outset, making studies of effects of individual fungi easier. They also allow the study of genetic interactions, since replication of genotype can be achieved.

A study of the mycorrhizal status of four clones of Sitka spruce was initiated when it was noted that there appeared to be differences in rooting density among plants in an experiment on biomass production (P. Biggin, Forestry Commission, pers. comm.), and measurements were made to investigate the role of mycorrhizae in this phenomenon.

Materials and Methods

Ortets of Sitka spruce, all open-pollinated half-sibling progeny of a tree in Glentress Forest, Scotland, had been rooted under mist and then transplanted for 18 months to the Forestry Commission's Bush Nursery, near Edinburgh. Similarly rooted cuttings that had recently been removed from the mist beds were non-mycorrhizal, and it may be assumed that the plants examined in this study were also without symbionts when transplanted.

The entire rooting systems were first examined under a dissecting microscope to assess the general level of colonization by mycorrhizal fungi, and to determine the morphological types of mycorrhizae present. Random samples of roots were then taken from four replicate ramets from each clone. The total length of each short root was measured, and counts made of the number of mycorrhizal tips and the number of fresh scars on the long root (which was taken to indicate the loss of a short root during processing).

Examination of the data showed that it was not normally distributed, and had inhomogeneous distribution. No suitable transformation could be found to normalize the data, so non-parametric methods were used in the anaysis, a Wilcoxon test being applied to the ranked data.

Results and conclusions

All short roots on the transplants were ecto-mycorrhizal. Two mycorrhizal types were recognized. Both were simple (monopodial) to elongate-short-branched. These were designated M51 and M52, respectively, and samples were preserved in 3.5% gluteraldehyde solution for future examination. Type M51 was clay-buff to sienna in color, with creamy tips. The mantle was thick and the Hartig net shallow, hardly penetrating beyond the first layer of cortical cells. Type M52 varied in color from sienna through dark brick to chestnut, and also had creamy tips. The mantle on this second mycorrhizal type was very thin (though complete), and the Hartig net was well-developed, penetrating almost to the endodermis.

Significant differences were noted for a number of parameters, and these are diagrammatically summarized in Figure 1. When these differences were examined, the reasons for the observed differences in root form became clear. They were all the result of one clone, clone 163, having higher values than the others, which did not differ significantly in any respect. Compared with the others, clone 163 had almost twice as many mycorrhizae present, and these mycorrhizae had more tips. They also had increased overall length. Both types of mycorrhiza tended to be more branched on clone 163 than on the other clones, but they behaved somewhat differently in relation to the clones. M51 mycorrhizae branched more in clone 163 than in the others, but were not increased in overall length. In contrast, in clone 163, M52 mycorrhizae were much longer in addition to having increased branching when compared with the others.

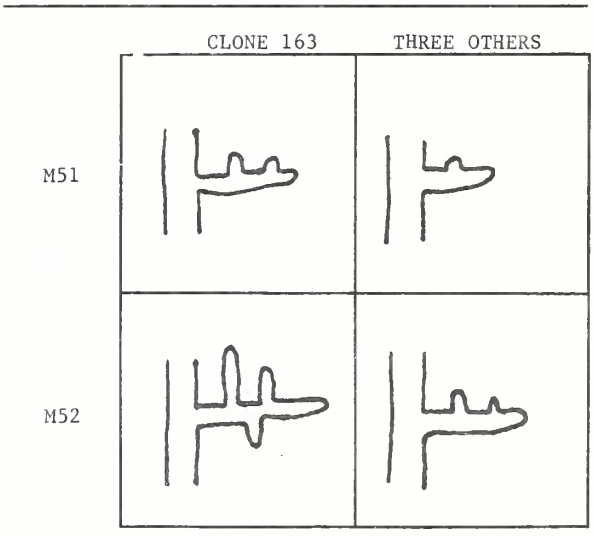

Figure 1: Diagrammatic representation of the mycorrhizal differences found between clone 163 (Sitka spruce) and three other spruce clones.

These differences show that there is clonal variation in response to mycorrhizal fungi among Sitka spruce progeny. Further work is in progress to investigate other clones, and to examine the possibility of using mycorrhizal characteristics as one criterion for selection of clones for testing in the forest.

ROOT MORPHOLOGY OF INOCULATED, CONTAINER-GROWN PINE SEEDLINGS INFLUENCES SPREAD OF *PISOLITHUS* TO EGRESSED ROOTS AFTER PLANTING

By

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Keywords--*Pinus taeda*, ectomycorrhizae

Introduction

Root morphology of container-grown loblolly pine (*Pinus taeda* L.) seedlings influences lateral root egress and spread of *Pisolithus tinctorius* (Pers.) Coker and Couch (Pt) after outplanting (Ruehle 1983). The growth performance of container-grown southern pine seedlings with Pt ectomycorrhizae compared poorly with similar bare-root seedlings outplanted on routine reforestation sites in Arkansas and Oklahoma (Ruehle and others 1981). This variance in field performance may be explained by differences between patterns of lateral and short root development and spread of Pt on these two types of planting stock.

Studies were established in microplots to determine the orientation of new lateral roots of bare-root and container-grown loblolly pine seedlings and the spread of Pt on new laterals after planting in forest soil.

Methods and Materials

Container seedlings were inoculated and produced in the greenhouse (14 weeks). Bare-root seedlings (1-0) were inoculated and produced in microplots. Sixty wood-framed microplots, 122 X 61 X 40 cm, were filled with loamy sand obtained from a mixed pine-hardwood stand. Two seedlings of the same treatment and stock type were hand planted at even spacing in the middle of each plot. Treatments were assigned to plots in a randomized block design of five replicates.

Six weeks after planting and every four weeks thereafter (5 sampling periods; 20 seedlings/period), seedlings were excavated. When excavated, each root plug or original root system was visually divided into three horizontal zones. The number of first-, second-, and third-order lateral roots emerging from each zone, the length of each lateral root, the number of Pt ectomycorrhizae present, and the greatest horizontal distance from the plug or original root system that Pt ectomycorrhizae had developed were recorded.

Results and Discussion

The pattern for lateral root growth from original root systems of bare-root seedlings was different from container-grown seedlings. After the first sampling period, significantly more roots emerged from the middle 4-cm zone on bare-root seedlings than on container-grown seedlings. Because of this greater development in the middle zone, the total new root development (number emerging from all zones) by the fifth sampling period was 66 percent greater on bare-root seedlings compared to container-grown seedlings. This increase in the middle zone also increased the tendency for more horizontal lateral root growth in the upper 10 cm of the soil profile.

By the fifth sampling period, Pt ectomycorrhizae on container-grown seedlings had spread horizontally on egressed roots an average of 17.5 cm, a distance comparable to that found on similar seedlings planted in nonfumigated soil in a previous study (Ruehle 1983). On bare-root seedlings, this horizontal spread of Pt (25.9 cm) was significantly greater.

General observations made at each sampling period found more Pt ectomycorrhizae on egressed laterals in zones above 10 cm than on egressed laterals lower in the soil profile. The problem of Pt spread in forest soil is much less on bare-root seedlings than on container-grown seedlings, probably, in part, because of different root configuration at planting and subsequent lateral root development. During the early months after planting, the reduced number of new laterals and spread of Pt to these roots on container-grown southern pine seedlings compared to bare-root seedlings probably accounts for the general lack of increased growth response of Pt-inoculated container-grown seedlings planted on routine sites.

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PERFORMANCE OF ECTOMYCORRHIZAL SITKA SPRUCE SEEDLINGS OUTPLANTED IN SE ALASKA

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Keywords--Hebeloma crustuliniforme, Laccaria laccata, Cenococcum geophilum

Introduction

Seedlings used for reforestation after timber harvest on the Tongass National Forest in southeast Alaska are grown in containers at the USDA Forest Service nursery in Petersburg, Alaska. Working at this nursery, Shaw and others (1, 2) have established Hebeloma crustuliniforme (Bull. ex St. Amans) Quel, Amanita muscaria (L. ex Fr.) Pers. ex Hooker, Laccaria laccata (Scop. ex Fr.) Berk and Br., and Cenococcum geophilum Fr. as ectomycorrhizae on Sitka spruce (Picea stichensis (Bong.) Carr.) seedlings. Survival and growth of these mycorrhizal seedlings, 2 years after planting on specific microsites in a clearcut near Pavlof Harbor, Chichagof Island, are presented here.

Methods

After old-growth forests are clearcut in southeast Alaska, the land typically consists of a mosaic of distinct microsites. We have described soil physical, chemical, and microbial properties for four common types of microsites in such clearcuts (rotten wood, exposed mineral soil, undisturbed duff, and undisturbed duff on the north side of a large stump) (3, 4). In this study, we outplanted Sitka spruce seedlings colonized by H. crustuliniforme, C. geophilum, and L. laccata, and a noninoculated control onto these microsites.

When seedlings were planted (October 1981), percentage of short roots colonized by each test fungus averaged 63% for H. crustiliniforme, 12% for C. geophilum, and 81% for L. laccata. Twenty examples of each microsite type were located and five of each type randomly chosen for planting with each fungal treatment. Seven seedlings were planted on each site and their total height and root collar diameter measured annually. Two year height and diameter increments were calculated from these measurements.

Mean growth of the seven seedlings on each site was used as the experimental unit in a two-way analysis of variance (ANOVA) performed to test if seedling growth was affected by fungal treatment or microsite type. If either main effect was significant, then the Bonferroni test was used to determine which pairs of site types or fungal treatments differed from one another. All differences were judged to be significant at P=0.05.

Results

At planting there was a significant difference in seedling size with all treatments differing from one another in height (Table 1). Seedlings colonized by C. geophilum were tallest and those colonized by H. crustuliniforme were shortest. Seedlings colonized by H. crustuliniforme also had a significantly smaller root collar diameter than all other types.

Over 95% of the seedlings were alive after 2 years. The most common cause of mortality was from frost heaving in exposed mineral soil. Total seedling height and diameter after 2 years did not differ significantly by microsite type; however, both measurements were significant by fungal treatment. Patterns of seedling height were the same as at planting--those colonized by C. geophilum were tallest and those colonized by H. crustuliniforme were shortest (Table 1). Although the ANOVA showed a significant effect of fungal treatment on diameter, variances were such that the Bonferroni test did not distinguish any significant differences between treatment pairs. Seedlings colonized by L. laccata and H. crustuliniforme, however, were about 13 mm smaller in diameter (53-54 mm) than controls or seedlings colonized by C. geophilum (63-64 mm).

Even though there was a significant difference among fungal treatments in seedling size at planting and after 2 years, there were no significant differences in 2-year height or diameter increments (Table 1). Expressing growth as a percentage of initial height or diameter showed that seedlings colonized by H. crustuliniforme had the greatest increase in size and those colonized by L. laccata the least. Mean percentage increases in height and diameter were, respectively, 46% and 100% for controls, 39% and 110% for C. geophilum, 28% and 65% for L. laccata, and 62% and 117% for H. crustuliniforme.

Discussion

Shaw et al. (2) suggested that slightly smaller seedlings successfully colonized with beneficial fungi may, when planted, outperform larger, nonmycorrhizal seedlings. These data suggest that height and diameter increments can be similar for smaller and larger seedlings and, thus, height differences apparent at the time of planting persist. The proportional increases in size for seedlings colonized by certain mycorrhizal fungi, however, may be greater than controls, as they were here for seedlings colonized by H. crustuliniforme, or less than controls, as they were here for seedlings colonized by L. laccata. We are continuing to monitor growth of these seedlings and are currently evaluating their relative nutrient status and mycorrhizal colonization levels.

Table 1. Mean seedling heights at planting (1981) and after 2 years growth (1983).

Year	Fungal treatments[1]			
	C. geophilum	L. laccata	H. crustuliniforme	Control
	(seedling height, cm)			
1981	23.1 a	18.7 b	13.4 c	21.1 d
1983	32.1 a	23.9 b	21.7 b	30.8 a
2-yr inc.	10.0 a	5.2 a	8.3 a	9.7 a

1/Within any one row, values followed by different letters differ significantly (P=0.05).

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EFFECTS OF FERTILIZATION AND FUNGAL STRAIN ON ECTOMYCORRHIZAL DEVELOPMENT OF SITKA SPRUCE SEEDLINGS

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Keywords--Laccaria laccata, Thelephora terrestris, "E-strain", forest, nursery

Introduction

In forest soils, mycorrhizae develop in an environment containing much lower concentrations of nutrients than occur in nursery soils; yet certain mycorrhizal fungi commonly occur in both environments. For such fungi, the relative ability of isolates obtained from a forest or nursery soil to form mycorrhizae on inoculated seedlings is not clearly understood.

We inoculated containerized Sitka spruce seedlings with either Laccaria laccata (Scop. ex Fr.) Cooke, Thelephora terrestris (Ehrh.) Fr., or the "E-strain" fungus--all species that readily form mycorrhizae on Sitka spruce. Two isolates were used for each species, one obtained from a nursery soil and one from a forest soil. Objectives were to test effects of different fertilization levels on mycorrhizal formation, and to see if inoculation and/or fertilizer regime affected seedling growth.

Methods

Inoculum of each isolate was mixed with a peat potting medium in ratios varying from 1 part inoculum to 30-45 parts potting medium. Nine paper pot trays with individual cell volumes of 62.5 cm^3 were filled with each of the 7 potting mixtures (6 fungi and a control), using one row of 8 cells for each mixture.

Into each cell, a 12 wk-old Sitka spruce germling was transplanted and trays were randomly located on a glasshouse bench. Trays were fertilized weekly with liquid applications of a soluble 19-19-19 (N-P-K) fertilizer, 3 trays with each of the following concentrations: 0.7 g/l, 1.4 g/l, and 2.8 g/l. These fertilization levels are respectively equivalent to 3.05, 6.1, and 12.2 g per m^2 of bench surface area.

Seedlings were measured after 14 wks for percentage of short roots colonized, height, shoot wt, root wt, and nutrient content of foliage (N, P, K & Mg). Data were subjected to an analysis of variance (ANOVA), testing effects of fertilization level and fungus type on percentage short root colonization, root wt, shoot wt, root wt-shoot wt ratio, and nutrient content of foliage. All differences were judged significant at P=0.05. Where differences were significant in the ANOVA, Tukey's test was used to identify which levels of fertilization and which fungal types differed from one another.

Results

The percentage of short roots colonized was significant by fungal type, but not by fertilizer level. Isolates of the same fungus did not differ significantly from one another in the percentage of short roots colonized, although the forest isolate of T. terrestris had slightly higher levels of short root colonization than did the nursery isolate. Even though fertilizer level was not significant in the ANOVA, the nursery isolate of L. laccata and both isolates of T. terrestris had higher levels of short root colonization at the moderate level of fertilization than at the high level, as follows:

Isolate*	Fertilization level			
	Low	Medium	High	Average**
	(% short root colonization)			
Control	65	45	60	57 a
L. laccata (R-68)	83	76	58	72 ab
L. laccata (S-14)	77	71	70	73 ab
E -strain (R-66)	100	99	98	99 b
E -strain (R-69)	100	98	99	99 b
T. terrestris (R-67)	84	86	59	76 ab
T. terrestris (R-34)	98	97	66	87 ab

* Within each fungal species the first isolate came from a nursery and the second from a forest.

**Values with no small-case letter(s) in common differ significantly from one another.

Total height, shoot wt, and root wt were significant by fertilizer level, but not fungal type. Seedlings reared at the low level of fertilization were consistently smaller than those reared at moderate and high levels, but seedlings grown at moderate and high levels did not differ in size from one another, as shown below:

Measurement	Fertilization level		
	Low	Medium	High
	(mean values *)		
Height (cm)	22.4 A	29.1 B	29.3 B
Shoot wt. (g)	0.80 A	1.25 B	1.34 B
Root wt. (g)	0.12 A	0.18 B	0.16 B
Rt/Sh ratio	0.13	0.17	0.16
Total wt. (g)	0.92 A	1.43 B	1.50 B

* Within any one row, values followed by different letters differ significantly.

Levels of N, P, K, and Mg in foliage were significant by fertilizer level, but not by fungal type. For each element, nutrient concentrations at the low level of fertilization were significantly less than at the moderate level, which was significantly less than at the high level.

Discussion

Our high level of fertilization approximates that used by the USDA Forest Service in Alaska for rearing containerized Sitka spruce seedlings. These data suggest that seedlings of a similar size can be grown with half as much fertilizer. Even though this reduced level of fertilization did not significantly increase short root colonization by mycorrhizal fungi, colonization levels were equivalent or higher at this moderate level than at the higher level. That fertilization levels did not effect levels of short root colonization contrasts with results for C. geophilum on Sitka spruce (2), but agrees with results for L. laccata on other conifer seedlings (1). It was surprising that no significant differences existed in levels of short root colonization between the two isolates for any species. Perhaps the ability of these fungi to rather quickly colonize a relative high proportion of a seedling's short roots, regardless of the environment's nutrient status, accounts for their common occurrence in forest and nursery soils.

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MYCORRHIZAL FORMATION ON CONTAINERIZED SEEDLINGS IN THE INTERMOUNTAIN REGION

By

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Keywords--Pinus monticola, Pinus ponderosa, Pseudotsuga menziesii, Hebeloma crustuliniforme, Laccaria laccata, Pisolithus tinctorius, Cenococcum geophilum, host specificity

Introduction

Practices used in growing containerized conifer seedlings can inhibit mycorrhizae. Transplanting shock and growth reduction are more likely prior to significant mycorrhizal development on newly planted seedlings. Containerized seedling vigor may be improved with controlled inoculation with selected mycorrhizal fungi.

Successful artificial inoculation requires infection of a high proportion of short roots. Mycorrhizal synthesis trials on western conifers have produced a broad range of infection success.

The objectives of this study were twofold: to determine whether containerized seedlings grown under Potlatch Corporation seedling production conditions would form mycorrhizae; and to determine which available fungal isolate more readily formed mycorrhizae on three species of Intermountain region conifers.

Methods and Materials

Mycorrhizal inoculum as fungal mycelium incorporated in peat-vermiculite was obtained from the following sources:
Pisolithus tinctorius - from the Institute of Mycorrhizal Research and Development, USFS, Athens, GA.
Pisolithus tinctorius - MycoRize® from Abbott Laboratories, Chicago, IL.
Pisolithus tinctorius and Laccaria laccata from Butler County Mushroom Farm, Worthington, PA.
Pisolithus tinctorius and Hebeloma crustuliniforme - from the University of Washington, Seattle, WA.
Laccaria laccata, Cenococcum geophilum, and Hebeloma crustuliniforme - from the Forestry Sciences Laboratory, USFS, Corvallis, OR.

Inoculum was mixed into peat-vermiculite as 10% of the volume added to 16 cm^3 styroblock cavities. Pseudotsuga menziesii, Pinus ponderosa, and Pinus monticola were seeded in spring and cultured as per standard greenhouse practice which included application of soluble fertilizers and pesticides during the March to November growing season.

A 10% randomized sample of each host species-inoculum source treatment combination was collected after seedlings were dormant in the fall. Shoot height, stem caliper, root and shoot dry weights, and percent mycorrhizal short roots were determined.

Results and Discussion

On all three conifer species tested, mycorrhizae formed more readily with H. crustuliniforme (Table 1). Seedlings infected with H. crustuliniforme were also smaller than noninoculated controls (Table 1). It is not known if this size differential will influence growth in the field.

Table 1. Percent mycorrhizae infection and (shoot height) (mm) of one-year-old containerized conifers.

Inoculum Source	Pinus monticola	Pinus ponderosa	Pinus menziesii
P.t.	26 (138)	34 (144)	30 (274)
L.l.	51 (112)	24 (121)	20 (223)
C.g.	46 (114)	16 (111)	23 (188)
H.c.	82 (114)	85 (123)	65 (192)
Control	<1 (127)	7 (134)	<1 (269)

No other greenhouse synthesis of mycorrhizae on P. monticola is known to us, and as Fig. 1 indicates, abundant mycorrhizae can be induced to form on this commercially important species.

Figure 1. H. crustuliniforme formation on container-grown P. ponderosa, P. menziesii, and P. monticola.

EFFICIENCY IN A FOREST NURSERY OF AN INOCULUM OF AN ECTOMYCORRHIZAL FUNGUS PRODUCED IN A FERMENTOR AND ENTRAPPED IN POLYMERIC GELS.

by

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Keywords - Picea abies - Pseudotsuga menziesii - Hebeloma cylindrosporum - fermentor polymeric gels.

Introduction

Pure culture inocula of ectomycorrhizal fungi are usually produced on a vermiculite peat mixture. They can also be prepared by cultivating the fungus in a fermentor and entrapping it in a polymeric gel as proposed by Dommergues and al. (1979) for bacteria and Jung and al. (1981) for different microorganisms.

Effectivness of Hebeloma cylindrosporum in forming ectomycorrhizae in nursery from different types of inoculum was tested on spruce (Picea abies L.) and Douglas fir, Pseudotsuga menziesii (Mirb.).

Materials and methods

We have prepared different types of inoculum of Hebeloma cylindrosporum as follows :

A. microgranules: mycelium included in an alginate gel and mixed with macroporous silica. 10 g of mycelium per square meter (dry weight)
B. idem - 5 g of mycelium per square meter
C. microgranules : dead mycelium included in alginate and mixed with silica-10 g of dead mycelium per square meter (dry weight)
D. microgranules : mycelium included in alginate and mixed with sepiolite 10 g of mycelium per square meter (dry weight)
E. pellets : mycelium included in alginate. Inoculum dried to a moisture content of 30 %
F. pellets : mycelium included in alginate. Inoculum dried to a moisture content of 12 %
G. pellets of mycelium coming directly from the fermentor
H. no mycelium ; only alginate and macroporous silica
I. mycelium grown in 2 liters volume of a vermiculite and peat moss mixture saturated with a nutrient medium
J. mycelium grown in 2 liters volume of a vermiculite and peat moss mixture saturated with a nutrient medium and leached with water after incubation
K. no mycelium : only the vermiculite and peat moss mixture saturated with a nutrient medium
L. no mycelium ; only the vermiculite and peat moss mixture saturated with a nutrient medium and leached with water
M. control (nothing).

The experiment was installed in a ten years old nursery with a sandy soil, a pH of 5.5 and a relatively high level ofmineral elements. The soil was disked with methyl bromide in early April. No fertilizer was applied. 152 test plots of one square meter were installed. The experiment was a randomized complete design with four replicates for each treatment. Six months after inoculation, in each plot, twenty seedlings were chosen at random and lifted. The degree of ectomycorrhizal development was estimated visually for each seedlings and for two ectomycorrhizal fungi : Hebeloma cylindrosporum which was inoculated, and Thelephora terrestris which had contaminated all the plots during the growing season. The height of one hundred seedlings was measured per plot.

Results

The entrapped mycelium becomes ineffective when the inoculum is dried to a moisture content of 30 %. Six types of inocula consistently produced Hebeloma ectomycorrhizae. The degree of ectomycorrhizal development is not significantly different between the different types of inoculum.
But, compared to the peat vermiculite inoculum, the mycelium produced in fermentor significantly stimulates seedlings growth, especially when it is entrapped in polymeric gels. Growth stimulation seems to be not related with Hebeloma ectomycorrhizal development.
The incidence of naturally occuring ectomycorrhizal fungi (Thelephora terrestris) decreases sharply in the treatments where Hebeloma cylindrosporum has formed mycorrhizae.
This method of producing mycelium in fermentor and entrapping it in a polymeric gel seems to be a suitable way for producing large quantities of commercial inoculum.

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FIELD RESPONSE OF PINUS SPECIES INOCULATED WITH ECTOMYCORRHIZAL FUNGI IN NIGERIA

By

S. A. Ekwebelam and M. A. Odeyinde

Key words - Pinus oocarpa, P. caribaea, Cenococcum, Pisolithus, Rhizopogon, Suillus, Afaka, Jere, Umuahia.

Introduction

The introduction of Pinus species into Nigeria is well documented (Madu, 1967). Among the tropical species, although P. caribaea Morelet and P. oocarpa Schiede have adapted most successfully for large scale afforestation, factors such as the mycorrhizal association still appear to be critical, hence the present study.

The present work examines the effect of different mycorrhizal fungi on growth and yield of P. caribaea var. bahamensis Barr. and Golf. and P. oocarpa Schiede seedlings in the nursery, and subsequently in the field in different habitats.

Methods and Materials

Seedlings of P. caribaea var. bahamensis (PCB) Barr. & Golf. and P. oocarpa Schiede inoculated with pure grain cultures of ectomycorrhizal fungi Cenococcum geophyllum (Fr.), Pisolithus tinctorius (Pers.) Coker & Couch., Rhizopogon luteolus Fr. & Nordh., Suillus bovinus (L. ex Fr.) O. Kuntze, S. granulatus (L. ex Fr.) O. Kuntze, S. luteus (L. ex Fr.) S. F. Gray and Thelephora terrestris (Ehrh.) Fr. were grown for 20 weeks in the nursery. The seedlings were subsequently outplanted in the fields at Afaka (Northern Guinea Savanna), Jere (Jos Plateau) and Umuahia (High Forest), and assessed for survival, height and stem growth, and volume over bark (yield) at age 5½ years.

Results and Discussion

The results indicate that large differences in stimulation of plant growth can occur following inoculation with different ectomycorrhizal fungi in the nursery, and subsequently in the field in different habitats, several years after planting. In the nursery, seedlings of PCB and P. oocarpa inoculated with S. luteus and S. bovinus, respectively were consistently taller than those of the other fungi, including the controls over the growing period. In the field at Afaka, although R. luteolus and P. tinctorius significantly improved the survival of PCB and P. oocarpa, respectively relative to the controls and some fungi, inoculation with T. terrestris resulted in greater height and stem growth, and significantly higher yield than the controls with both host species. At Jere, where seedling mortality varied from 30-100 percent, S. luteus gave consistently over 90 percent survivors with both hosts, and whereas P. tinctorius and the noninoculated controls produced the best height and stem growth, respectively with PCB, S. bovinus stimulated the growth and yield of P. oocarpa better than the other fungi, including the controls. At Umuahia, inoculation with C. geophyllum produced the best stem growth and yield with PCB. On P. oocarpa, inoculation with P. tinctorius, R. luteolus, S. luteus and T. terrestris significantly improved seedling survival in comparison with C. geophyllum and the controls, and S. luteus and R. luteolus respectively produced the best height growth and yield.

The improvements in growth brought about by some of the fungi under the nursery conditions did not manifest themselves under the different field conditions; nor was any particular fungus consistent in promoting growth and yield of both hosts in the different field locations. While these results suggest that the effects produced by any fungus depend to a certain extent on host species and environmental conditions, they also emphasize the impact of pure culture inoculation on pine establishment in Nigeria.

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INFLUENCE OF NITROGEN AND PHOSPHORUS FERTILIZATION ON ECTOMYCORRHIZAL FORMATION OF *QUERCUS ALBA* AND *Q. RUBRA* SEEDLINGS BY *PISOLITHUS TINCTORIUS* AND *SCLERODERMA AURANTEUM*

By

P. Beckjord, J. Melhuish, Jr., and M. McIntosh

Keywords--Ectomycorrhizae, nutrients, oak seedling production, basidiospore inoculation

Introduction

An excellent review on the mineral nutrition of ectomycorrhizae has been provided by Bowen (1973). Motivated by Bowen's work, Beckjord *et al.* (1980, 1983) investigated the effects of nitrogen fertilization on ectomycorrhizal formation of several oak species during seedling production. In the course of conducting those experiments, one fundamental question continued to surface; what was the optimum amount of nitrogen or phosphorus (or both in combination) that would be necessary to maximize ectomycorrhizal formation on oak seedlings.

Methods and Materials

Perlite growth medium inoculated with or without inoculum (62:1 v/v, respectively) of *P. tinctorius* (Pt) & *S. auranteum* (Sa) was added to containers (780 ml@). Inoculum consisted of 100 ml basidiospore/basidiocarp tissue (45 µm mesh) and 5L peat moss (No. 20 mesh). Germinated acorns of *Q. alba* and *Q. rubra* were planted and oak seedlings were grown in a greenhouse for 100 days. Nine seedlings of each oak species/inoculation treatment were fertilized with or without base nutrient solution by subirrigation Mondays, Wednesdays and Fridays, (Folwells and Krauss 1959), and flushed with distilled water on other days. During subirrigation fertilization, seedlings were also fertilized with 1, 10, 100, 200, or 400 ppm N supplied as ammonium nitrate (P fixed at 10 ppm) or 1, 5, 10, or 20 ppm P as potassium phosphate (N fixed at 100 ppm). Seedling percent(s) ectomycorrhizal short roots (PESR) was determined after the growth period.

Results and Discussion

Seedlings not inoculated with Pt and Sa were free of ectomycorrhizae. All inoculated seedlings receiving 0, 200, and 400 ppm N were not ectomycorrhizal, or nearly so (Table 1). The PESR by Pt followed a similar pattern for both oak species in relationship with the fertilizer treatments. PESR by Pt was maximized for both oak species when fertilized with 10 ppm N and 1 ppm P. PESR by Sa on *Q. alba* were negligible. However, PESR by Sa on *Q. rubra* was maximized with 10 ppm N and 5 ppm P.

This study provided two major results. First, ectomycorrhizal formation was inhibited with high levels of N and P and without N and P. Ectomycorrhizal formation was maximized on both oak species by both fungal species with fertilization of 10 ppm N (P fixed at 10 ppm). The second result was that symbiotic compatibility [e.g. host specificity (Molina 1979)] between *Q. alba* and Sa was not demonstrated.

Table 1. Percent(s) ectomycorrhizal short roots and related data for *Quercus alba* and *Q. rubra* seedlings inoculated by *Pisolithus tinctorius* and *Scleroderma auranteum* and fertilized with various levels of nitrogen and phosphorus.

Fertilizer treatment		Inoculation treatment: *Quercus alba* Pt PESR	Pt N/S	Pt S.D.	Sa PESR	Sa N/S	Sa S.D.
0		0	0/9	-	1	0/6	2
1 N	10P	20	7/7	15	3	5/5	4
10 N	10P	30	8/8	24	3	9/9	4
100 N	10P	1	3/8	3	2	2/6	2
200 N	10P	0	0/8	-	0	1/8	-
400 N	10P	0	0/8	-	0	1/7	-
1 P	100N	29	7/9	24	3	6/7	5
5 P	100N	10	7/9	9	1	2/7	2
10 P	100N	9	3/9	18	0	0/8	-
20 P	100N	6	5/8	14	0	0/9	-
		Quercus rubra					
0		0	0/9	-	0	0/9	-
1 N	10P	10	9/9	11	4	5/9	7
10 N	10P	25	8/9	20	38	9/9	19
100 N	10P	5	6/9	8	1	2/9	3
200 N	10P	0	0/9	-	0	0/9	-
400 N	10P	0	0/9	-	0	0/9	-
1 P	100N	19	5/8	22	11	4/9	17
5 P	100N	5	5/9	8	15	5/9	27
10 P	100N	0	0/8	-	4	4/9	4
20 P	100N	0	0/9	-	4	2/9	8

Fertilizer treatment 0 = control fertilization (no N or P or base nutrient solution), N = nitrogen, P = phosphorus, PESR = percent ectomycorrhizal short roots, N/S = number of ectomycorrhizal seedlings out of number surviving, S.D. = standard deviation, Pt = *Pisolithus tinctorius* and Sa = *Scleroderma auranteum*.

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INCREASED SURVIVAL OF LONGLEAF PINE WITH PISOLITHUS TINCTORIUS AND BENOMYL

By

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Keywords--Pinus palustris, Brown-spot needle blight, Scirrhia acicola

Introduction

Regeneration of longleaf pine, (Pinus palustris Mill.), depends upon procedures that promote survival, stimulate growth, and control Brown spot needle blight (Scirrhia acicola (Dearn.) Siggers). Theoretically, survival could be improved by using seedlings with ectomycorrhizae of Pisolithus tinctorius (Pers.) Coker and Couch (Pt) if protected from brown spot.

Longleaf seedlings with and without Pt ectomycorrhizae were treated with benomyl (a systemic fungicide) and then outplanted on high hazard disease sites in Louisiana (LA), Mississippi (MS), Alabama (AL), and Florida (FL). Objective was to improve survival by stimulating growth concurrently with controlling brown spot.

Methods and Materials

Seedlings were inoculated with vegetative inoculum of Pt in the seedbed by the "row-drill soil-injected technique." After dormancy and lifting, Pt seedlings were graded to a minimum Pt index of 50 (mean = 61).

Identical tests were installed in the four states. A total of 500 Pt and 500 Pt-free seedlings (with abundant natural ectomycorrhizae) were machine planted at sites in January 1982 (Fig. 1). Seedlings of each group were subdivided into four benomyl treatment groups. Seedling roots were moistened in water and then shaken in a plastic bag containing various mixtures of Kaolinite clay and benomyl (0, 5, 10, and 20 percent a.i. by volume).

Twenty-five seedlings of eight treatments were planted in each of five blocks at the four sites. They were planted 3-ft. apart in rows spaced at 10-ft. intervals. Blocks were separated by a 20-ft. buffer zone.

Sample seedlings were lifted from each treatment block combination and assessed for occurrence of Pt and other ectomycorrhizae in November 1982. Second year survival (November 1983) was analyzed by an analysis of variance and compared by Duncan's multiple range test (Table 1).

Results and Discussion

Survival at a site was significantly increased by both the Pt ectomycorrhizae and benomyl root dip treatment. Pt seedlings had higher survival rates than Pt-free seedlings; 21% more at LA, 24% more at MS, 6% more at AL, and 22% more at FL. Survival was greatest with the 5 and 10% benomyl rates for both Pt and Pt-free seedlings. Control seedlings (Clay dip) without Pt had significantly lower survival rates at all sites.

The lower survival difference (6%) at the AL site was due to the natural occurrence of P. tinctorius which did not occur at other sites. No.Pt fruiting bodies were noted on any site in 1982 or 1983. Other ectomycorrhizae were observed on roots of all sites after one year in the field. For benomyl, decreases in survival at the 20% a.i. dosage rate indicated a phytotoxic effect. The 5% benomyl treatment of Pt seedlings was optimal for maximum survival.

Table 1. Effect of P. tinctorius on survival of longleaf pine seedlings in the field.

Treatment[1]	Location of planting				
	LA	MS	AL	FL	Mean
Pt-5% Ben.	92a	89a	79a	91a	88
Pt-10% Ben.	91a	84a	84a	83ab	86
Pt-20% Ben.	76abc	83a	72ab	63cd	74
Pt-Clay	83ab	90a	57bc	90a	80
Mean	86	87	73	82	
No Pt-5% Ben.	75abc	76ab	66abc	70bc	72
No Pt-10% Ben.	69bcd	71ab	81a	62cd	71
No Pt-20% Ben.	55d	59bc	73ab	49d	59
No Pt-Clay	59cd	53c	47c	57cd	54
Mean	65	63	67	60	

1/Seedlings with or without P. tinctorius ectomycorrhizae were dipped in various V/V mixes of benomyl (a.i.) and clay prior to planting.

2/For each site, values having same letter are not significantly different (P=0.05).

Figure 1. Pt-free seedlings (left) and Pt inoculated seedlings (right) of longleaf pine.

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MYCELIUM DERIVED FROM SCLEROTIA AS A SOURCE OF INOCULUM FOR ECTOMYCORRHIZAE

By

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Keywords--Pisolithus tinctorius, Paxillus involutus, germination

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Introduction

Ectomycorrhizal fungi for growth in pure culture are generally isolated from sporophores of Basidiomycetous fungi (Trappe, 1962). Although some researchers have isolated ectomycorrhizal fungi from sclerotia collected in the field (Trappe, 1969; Laiho, 1977; Dennis, 1980), none has shown specifically that mycelium derived from sclerotia is an effective inoculum. The structure and physiology of sclerotia indicate that they would make ideal organs for long-term storage of genetic traits of specific fungal strains. The objective of this study is to assess the effectiveness of sclerotium-derived mycelium of Pisolithus tinctorius Coker and Couch and Paxillus involutus (Batsch) Fr. in establishing ectomycorrhizae in pine.

Materials and Methods

Cultures of fungi were maintained on modified Melin-Norkrans (MNM) agar medium (Fortin et al., 1980). Plugs of mycelium (10 mm in diameter) were taken from actively growing cultures, and transferred to fresh medium for 3 days to permit regeneration of hyphae on margins of the plug. Plugs were then transferred to plastic growth pouches containing seedlings of Pinus resinosa and Pinus strobus (Fortin et al., 1980). Four to five plugs were placed in each pouch, about 5 mm from actively growing lateral roots. Ten ml of MNM nutrient solution was added to the pouch at this time. A further 10 ml of nutrient solution was added after mantle formation. Sclerotia formed in the pouches after 11-15 days were collected and stored on filter paper in petri dishes for up to 30 days. Before culturing, sclerotia were soaked overnight in distilled water, surface sterilized in 30% H_2O_2 for 20 minutes, rinsed in sterilized H_2O and placed on MNM agar. After two weeks of growth, plugs were taken and placed in pouches containing pine seedlings as described above. Subsequently, infected short roots were fixed, resin-embedded, sectioned and stained with toluidine blue-O. To assess the reproducibility of these results all steps were repeated three times.

Results and Discussion

Regeneration of hyphae occurred between 2-6 days for P. tinctorius (Fig. 1) and 3-7 days for P. involutus. The sclerotium-derived mycelium of both species was successful in forming mantles on short roots of both species of pine (Fig. 2). Sections of short roots revealed, in all cases, a well developed Hartig net (Fig. 3). Mycelium from sclerotia of P. involutus grown in pure culture was also effective inoculum.

This study indicates that sclerotia can produce effective inoculum for the production of mycorrhizae. The possibility of using sclerotia directly as a source of inoculum is being studied. The usefulness of sclerotia as a means of storing fungal strains, as well as their possible role in practical forestry, makes them an obvious focus of research.

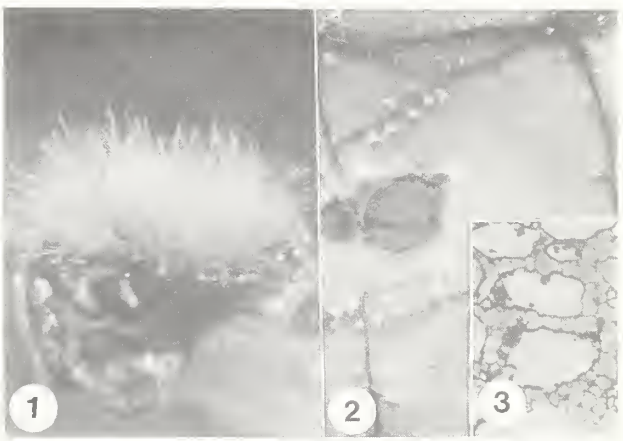

Figure 1. Germinating sclerotium of P. tinctorius.

Figure 2. Ectomycorrhizae on Pine formed by P. involutus.

Figure 3. Hartig net formed by P. involutus.

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EFFECT OF FERTILIZERS AND ECTOMYCORRHIZAL INOCULUM ON STUNTED DOUGLAS FIRS

By

I.R. Hall and E. Garden

Keywords--Pseudotsuga menziesii, field experiment, chlorosis

Introduction

Over the past few decades nurserymen have been made aware of the need to ensure that normally ectomycorrhizal plants are infected with a suitable mycorrhizal fungus. For example in 1958, Gilmour advised that chlorosis followed by stagnation which affected up to 80% of newly established Douglas fir (Pseudotsuga menziesii [Mirb.] Franco) that came from a nursery in the South Island of New Zealand, was due to a lack of mycorrhizal development caused by the "absence of proper inoculation, certain unfavourable nursery practices and possibly periodic anaerobic soil conditions". It was therefore of some concern that approximately 40% of Douglas fir seedlings planted in 1977 on the property of the second author, developed symptoms identical to those described by Gilmour. The experiment reported here was designed to investigate in the field possible causes for this poor growth and if the symptoms could be aleviated.

Methods and Materials

The Douglas fir plantation used in this study is 460 m above sea level at Avenal Station, near Millers Flat, Central Otago, New Zealand. Ectomycorrhizal plants had never grown on the site, the nearest ones being a few well established Douglas firs in a shelter belt approximately 1 km away. The area was previously occupied by run-out pasture which had not been topdressed in the previous five years. The soil had a pH of 5.3, 16 µg Olsen extractable P/ml soil and a P retention of 33%.

Because of the deficient levels of N, S and P in the foliage of the chlorotic and stunted trees (Table 1) and the possible lack of mycorrhizas on their root systems the treatments were: 1. Uninoculated or inoculated with c. 50 g of duff collected from under a Douglas fir shelter belt 2. No fertilizers, 15 g NH_4NO_3, 2 g $(NH_4)_2SO_4$ or 2 g $NH_4H_2PO_4$/plant.

The treatments were applied once to the roots of the stunted trees and their growth was monitored over a four year period.

Results and Discussion

All the nutrients significantly stimulated growth of the uninoculated trees. Inoculation also stimulated growth though this rendered the trees unresponsive to the fertilizers (Figure 1).

The data could be interpreted to suggest that available phosphorus, sulphur, and nitrogen levels in the soil at the plantation were deficient as was the mycorrhizal status of the stunted trees and that the alleviation of just one of these factors was sufficient to release the trees from their stunted condition. But by the end of the experiment phosphorus, sulphur and nitrogen in the uninoculated controls were generally higher than in all other treatments. Also the amounts of nutrients added in the fertilizers would not have been sufficient to support the non-mycorrhizal growth of trees with a dry weight in excess of 10 kg and containing for example about 0.5% nitrogen and 0.05% phosphorus. It was therefore concluded that the nutrients and the mycorrhizal inoculum helped redress a mycorrhizal deficiency brought about by low nutrient levels in the soil at the plantation (Heilman & Ekuan, 1980) though the absence of a suitable mycorrhizal fungus on the outplants may also have been a contributory factor.

Table 1. Nitrogen, sulphur and phosphorus concentrations (%) in the needles of chlorotic and stunted, and healthy Douglas firs

	Stunted	Healthy
N	0.47	1.75
S	0.04	0.13
P	0.11	0.17

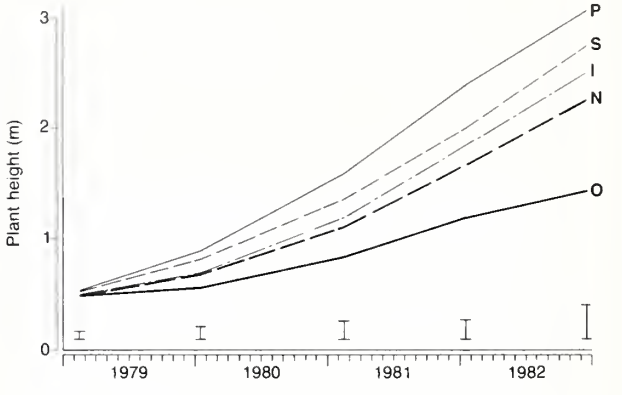

Figure 1. Heights of Douglas firs receiving no fertilizer and with inoculum (I) or no inoculum and plus nitrogen (N), sulphur (S) or phosphorus (P) or without fertiliser (O). Bars are SEMs.

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DEVELOPMENT OF METHODS FOR THE PRODUCTION OF MYCELIAL SLURRY INOCULUM

By: C.D. Boyle, K.L. Gunn and W.J. Robertson

Keywords--Mycelial Macerate, Viability Testing

Introduction

One of the major expenses with the application of ectomycorrhizal fungi is the inoculum. This is typically vermiculite-based, added to the rooting medium after leaching to remove nutrients. The fungus is thus nutrient starved until after germination when root exudation starts. Viability may decrease during this lag period, necessitating a large inoculum. If the inoculum were added after tree germination, a small inoculum might be adequate. We contend that a liquid inoculum (e.g. fragmented mycelium) would be most appropriate in this respect.

We have a program at the N.S. Res. Fdn. Corp. to develope a mycelial slurry inoculum. Our program consists of three parts: A. Growth of Fungi in Liquid Culture, B. Development of Techniques for the Maceration, Storage and Handling of Liquid Inoculum and C. Greenhouse and Field Testing. We present some of our results from part B here.

Methods and Materials

In preliminary experiments we found that most ectomycorrhizal fungi grew better on MMN liquid medium (Marx 1969) than on other common media, but the pH dropped to below 3. We found that inclusion of 0.01 M citrate maintained the pH above 4. (0.1 or 0.05 M inhibited growth.) We grew our fungi in MMN + citrate in either flasks or simple 6 L aerated culture vessels. Most fungi tested grow reasonably well.

Minimal viable mycelial fragment sizes were determined by passing a mixture of mycelium homogenized for 1 sec. and 30 sec. (wide fragment size range) through a series of sterile filters with 650, 220, 180, 150 and 120 µm mesh. The filtrate was transferred to flasks containing MMN + citrate. After 2 weeks at 25°C, treatments were assessed as positive if any growth was apparent.

Viability assays to quantify maceration and storage techniques were conducted on mycelium collected from 6 L culture vessels. This was homogenized at 1 g fresh wt/100 mL for 10 sec. in a Waring blender operated at "low". Ten fold dilutions were made up to 10^{-7}, in triplicate, using MMN medium as diluent. After 1 week at 25°C, tubes showing growth were used to calculate the most probable number of viable propagules. Before inoculation, 1 mL aliquots from each of the 10^{-3}, 10^{-4} and 10^{-5} dilution tubes were spread on MMN plates. Plates with between 10 and 200 colonies, after 1 week at 25°C, were used to estimate number of viable propagules (Plate Count). One mL aliquots were withdrawn from the 10^{-2} dilution tube and used to inoculate 50 mL MMN medium in flasks. Dry weight of mycelium (mg) after 1 week at 25°C was used as the Growth Index.

Results and Discussion

The fungi tested varied somewhat in their minimal sized viable propagules, although in all cases they passed the 150 µm mesh (Table 1). Small propagules would be physically advantageous. For example they could more readily percolate into the root zone.

Table 1. Minimal viable fragment size.

Filter mesh (µm)	P. tinct.	C. geo.	E. strain
650, 220, 180 or 150	+	+	+
120	+	+	-

The three viability assays showed similar major trends with Cenococcum geophilum. Minor differences are probably not significant. Viability had dropped markedly after 1 month storage. It was not dramatically affected by maceration or storage medium, or by storage temperature (Table 2). We also found little difference between preparations macerated before or after storage (data not presented).

Table 2. Effects of storage temperature and maceration medium on mycelial fragment viability using Cenococcum geophilum.

Assay technique		Homogenization medium	
A.	Before storage	$d\ H_2O$	MMN + citrate
	MPN	$>2.3 \times 10^5$	2.3×10^5
	Plate count	2.3×10^6	-
	Growth index	5.7	3.2
B.	After storage (1 month, 4°C		
	MPN	9.3×10^4	4.3×10^4
	Plate count	8.5×10^4	2.6×10^4
	Growth index	1.9	1.5
C.	After storage (1 month, 25°C		
	MPN	9.3×10^4	2.3×10^4
	Plate count	2.5×10^4	1.9×10^4
	Growth index	1.5	1.5

We are continuing this work to include other fungi. We hope to develope techniques for the production of mycelial macerates with high viability and infectivity.

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DYNAMICS OF MYCORRHIZAE AFTER INOCULATION IN NON-DISINFECTED NURSERY SOILS

By

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Keywords--Picea abies, Hebeloma cylindrosporum, Boletus edulis, Cenococcum geophilum, competition

Introduction

The growth of ectomycorrhizal fungi is strongly influenced by ecological and site factors, as the complicated distribution patterns and high host specificity of certain fungi show. The success of artificial inoculation consequently depends greatly on the ability of the inoculated fungus to adapt to the given site conditions.

The present study investigates the competitive strength of artificially inoculated mycorrhizal fungi in 3 non-disinfected nursery soils, with special attention to the interplay between inoculated and soil-borne fungi.

Methods and Materials

3 ectomycorrhizal fungi, Hebeloma cylindrosporum, Boletus edulis, and Cenococcum geophilum, were grown in liquid culture (MMN) and liquid inocula with known concentrations of living mycelial fragments were prepared (after Garbaye, 1983). Two-month-old, non-mycorrhizal spruce seedlings (Picea abies) were planted in rootrainers® with 3 non-disinfected nursery soils (EAFV, Rodels, Disentis), and immediately inoculated. 32 replicates each of 5000 (Dos. 1) and 50 000 prop./plant (Dos.2), with controls, were set up. The plants were grown on in a greenhouse under normal conditions of light and temperature, without fertilisation. Periodically, the rootrainers were opened and mycorrhizal formation was assessed. The frequency of the various types of mycorrhiza, both inoculated and soil-borne, was estimated on a five-graded scale and averaged for each set of replicates.

Results and Discussion

On all 3 soils, B. edulis and C. geophilum disappeared shortly after inoculation without having formed mycorrhizae. H. cylindrosporum developed rapidly (see Fig. 1), with the first mycorrhizae detectable after only 21 days. The level of infection reached maximum values (5 to 25 % according to soil type and inoculation dosage) within 4 to 7 weeks after inoculation. With the first appearance of soil-borne mycorrhizae, the level of infection sank more or less rapidly, depending on soil type. 24 weeks after inoculation, mycorrhizae of H. cylindrosporum were no longer detectable on the EAFV soil.
Thus even H. cylindrosporum appears completely or almost incapable of competing in the 3 soils investigated. Soil-borne mycorrhizal fungi seem to affect the competitive performance of the inoculated fungi decisively.

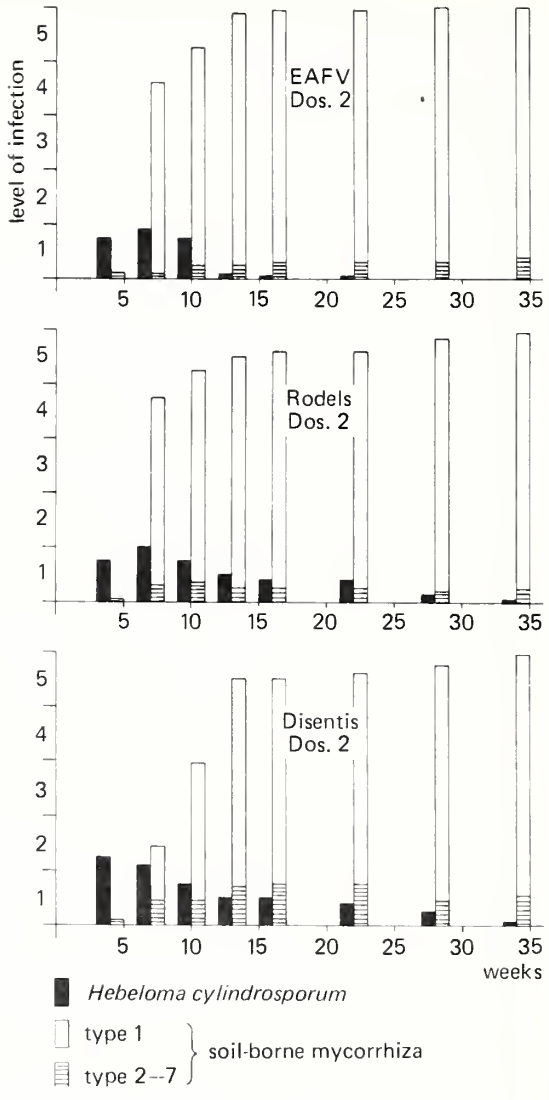

Figure 1. Development of H. cylindrosporum and soil-borne mycorrhizae after inoculation (50 000 prop./plant) on 3 soil types.

The inoculation dosage affected success very clearly: the higher the dosage, the higher the level of infection.

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INCREASED PASTURE GROWTH AFTER INOCULATING UNSTERILE SOILS WITH ENDOMYCORRHIZAL FUNGI

By

I.R. Hall

Keywords--Trifolium repens, Lolium perenne, Glomus, Gigaspora, growth responses, phosphorus, field experiments

Introduction

There is considerable interest in the possibility of exploiting the vesicular-arbuscular mycorrhizal (VAM) symbiosis (Abbott & Robson, 1982). However, despite a plethora of papers on VAM and the fungi which produce them, little field work has been published. Unfortunately in some of these papers techniques were employed which could not be applied on a farm scale or which make the results suspect (Abbott & Robson, 1982) such as the use of transplant techniques.

Four field experiments were therefore established using conventional agronomic practices to test the effect of introducing selected fungi (Hall, 1984) on the growth of oversown Trifolium repens L. (white clover) or white clover - Lolium perenne L. (perennial ryegrass) pastures.

Methods and Materials

The four sites chosen ranged in fertility from undeveloped, phosphorus-impoverished tussock grassland to high fertility alluvial flats. As far as practicable conventional agronomic practices were followed prior to and during sowing. The soils were otherwise untreated and contained the normal complement of indigenous VAM fungi. Infested soil pellets were used (Hall & Kelson, 1981) to introduce the fungi into the soils as this was considered more appropriate to pastoral farming than for example transplanting infected seedlings. It was shown in a previous experiment that the inocula contained not only the VAM fungi but also a filtrable agent that reduced the growth of white clover (Hall, 1984). However in these field experiments it was decided that the control pellets would not be inoculated with these filtrable agents so that any growth increments to VAM inoculation that did occur would be conservative estimates of what might be obtained in practice.

The four mycorrhizal treatments were a control where only the indigenous fungi would have been present, inoculated with Glomus mosseae, a mixed culture of G. tenue and G. pallidum or Gigaspora margarita which was replaced by Glomus macrocarpum in two of the experiments. There were four phosphorus treatments in each experiment, 0, 10, 20 and 50 kg P/ha applied as Ca $(H_2PO_4)_2.H_2O$, with 40 kg P/ha replacing 50 kg/ha on the two more fertile sites. These were applied 5 weeks after sowing, when it was assumed that mycorrhizas would have formed and again in each subsequent spring. In all the experiments a 4 x 4 factorial design was used which was laid out in six randomized blocks. Pasture growth was monitored by harvesting and weighing the herbage from one to nine times per year for a period of three years. No attempt was made to determine the proportion of roots infected by the indigenous or inoculant fungi as G. mosseae and G. tenue produced infections almost indistinguishable from those produced by some of the indigenous fungi.

Results and Discussion

Initially the introduced fungi tended to have little or no effect on dry matter yields but by the end of the first season there were significant responses to Gigaspora margarita, Glomus mosseae, Glomus macrocarpum or a mixed inoculum of Glomus pallidum and Glomus tenue. In the second season, dry matter yields were increased on the two tussock grassland sites by up to 760 kg DM/ha (c. 30%), 825 kg DM/ha on the moderately fertile site (14% - Table 1) and 640 kg DM/ha (5%) on the high fertility site. The growth responses tended to decrease with time probably owing to the spread of fungi from the inoculated to the control plots, though growth responses were still present at the end of the third year on two of the sites. Within each experiment the application of up to 50 kg P/ha per year did not reduce the size of the responses to inoculation. This and herbage chemical analyses suggested that a more efficient use of phosphatic fertilizers may have been only part of the reason for the responses to inoculation.

Table 1. Effect of applied P and inoculation on mean total dry matter yields (t/ha) in the second and third years on the moderately fertile site.

Inoculum	Applied P (kg/ha)	1980/81	1981/82
Control	0	4.94	4.28
	0-40	5.56	5.59
	10-40	5.76	6.02
Glomus mosseae	0	5.52	4.49
	0-40	6.32	6.15
	10-40	6.59	6.70
Glomus macrocarpum	0	5.04	3.66
	0-40	6.08	6.01
	10-40	6.43	6.79
Glomus tenue + Glomus pallidum	0	4.69	4.31
	0-40	5.78	5.92
	10-40	6.14	6.45
S.E.M.	0	0.308	0.400
	0-40	0.154	0.200
	10-40	0.178	0.231

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RESPONSE OF TROPICAL FORAGE PLANTS TO VA MYCORRHIZAL INOCULATION AND ROCK PHOSPHATE

By

S. R. Saif

Keywords--Stylosanthes, Pueraria, Andropogon, growth, mineral uptake, nodulation, Oxisol

Introduction

Most of the soils of the tropical America consists of Oxisols and Ultisols which are very low in phosphorus and has high P fixing capacity. At CIAT investigation is underway to search for alternate cheap fertilizer sources and the use of Rhizobium and vesicular-arbuscular mycorrhiza (VAM) for the better establishment and growth of tropical forage plants. The objective of this field experiment was to see if mycorrhizal inoculation increase the growth and mineral uptake of tropical forage plants and if rock phosphate, combined with mycorrhiza, is better than the rock phosphate alone.

Methods and Materials

The experiment was established at Carimagua (45°N, 71.5°W, 150 m elevation, 2.300 mm annual rainfall, 28°C mean temperature) in the Llanos Orientales of Colombia, South America. The plots were fertilized with low nutrient levels. The treatments applied were: uninoculated (NIL) with 0 P; inoculated (M) with 0 P; rock phosphate (RP), 20 kg P/ha; rock phosphate plus mycorrhiza (RP+M). A randomized complete block design with three replicates was used. Each plot measured 3x5 m and included five rows at 50 cm spacing.

Stylosanthes capitata CIAT 1315, Pueraria phaseoloides CIAT 9900 and Andropogon gayanus CIAT 621 seeds were sown in furrows. Legumes were inoculated with appropriate Rhizobium strains. A mixture of three mycorrhizal fungi i.e. Enthrophospora sp; Acaulospora sp and Glomus manihotis previously multiplied in pot cultures was used as inoculum. Approximately 200 g/liner m of soil and root debris was placed in furrows below the seeds. Three months after sowing 1st cut was made on the central 3 rows with an area of 4.5 m^2 and fresh and dry matter, mineral uptake and nodulation were evaluated.

Results and Discussion

Table 1 shows that fresh and dry matter production of all the plant species was significantly increased by mycorrhizal inoculation (M and RP+M), which implies that although these plants were infected with indigenous mycorrhizal fungi, the native fungi were not fully effective under the conditions used, and that the introduced mycorrhizal fungi were able to compete with the native ones. The low yield of inoculated (M) plants but with zero P compared to the RP+M plants further indicates that mycorrhizal inoculation has to be combined, in soils very low in P, with some addition of phosphate fertilizer to obtain the optimum yields. Table 2 shows that total mineral uptake of all the test plants was also increased significantly by mycorrhizal inoculation and the RP+M plants showed the highest uptake values. Table 3 shows that nodulation in Pueraria and Stylosanthes was also increased by mycorrhizal inoculation which clearly indicates the necessity of inoculating tropical forage legumes, if grown in low P soil, with effective mycorrhizal fungi for better nodulation and nitrogen fixation. The data clearly indicate that it is possible to increase yield, mineral uptake and nodulations of tropical forage plants, when grown in infertile soils, if they are inoculated with effective mycorrhizal fungi. This experiment is being continued to evaluate the longevity of the inoculation effect.

Table 1. Fresh (t/ha) and dry (kg/ha) matter of three forage plants grown in field in a Colombian Oxisol for 3 months.*

Treatments	Stylosanthes capitata		Pueraria phaseoloides		Andropogon gayanus	
	FM	DM	FM	DM	FM	DM
NIL	0.3a	69.7a	0.3a	74.6a	0.9a	272.7a
M	0.5b	126.2b	0.6b	150.9b	1.2b	376.6b
RP	1.5c	397.5c	2.5c	522.6c	7.2c	2280.0c
RP+M	3.0d	673.1d	4.3d	871.5d	9.7d	3046.0d

* FM, fresh matter; DM, dry matter; Different letters in a column represent significant differences (P<0.01).

Table 2. Total mineral uptake (kg/ha) of three forage plants grown in field in a Colombian Oxisol for 3 months.*

Species	Treatments	Total mineral uptake (kg/ha) N	P	K	Ca	Mg
Stylosanthes	a	2.43	0.10	1.38	0.37	0.28
	b	4.57	0.19	2.90	0.72	0.54
	c	17.30	0.94	9.67	3.89	1.74
	d	27.53	1.24	13.23	5.92	2.70
Pueraria	a	2.73	0.10	1.57	0.48	0.22
	b	4.84	0.15	2.74	0.85	0.40
	c	14.96	0.77	8.93	2.96	1.25
	d	24.39	1.33	13.58	5.05	2.33
Andropogon	a	4.59	0.23	4.12	0.85	0.40
	b	6.77	0.34	5.80	1.75	0.69
	c	32.54	2.05	26.10	12.16	5.76
	d	43.92	2.84	33.95	13.36	6.80

* a, NIL; b, M; c, RP; d, RP+M. All means in a column for each plant differed significantly (P<0.01).

Table 3. Number of nodules/plant of Pueraria and Stylosanthes grown in field in a Colombian Oxisol for 3 months.*

Treatments	Pueraria	Stylosanthes
NIL	6.53a	4.74a
M	12.27b	8.02b
RP	25.46c	14.99c
RP+M	46.00d	24.79d

* Different letters in a column represent significant differences (P<0.01).

RESPONSE OF TROPICAL FORAGE PLANTS TO TWO SOURCES OF POTASSIUM AND VA MYCORRHIZAL INOCULATION

By
S. R. Saif and J. G. Salinas

Keywords--KCl, K feldspar rock, Stylosanthes, Centrosema, Zornia, Andropogon, Panicum, Unsterilized Oxisol.

Introduction

The low-input strategy adapted by Tropical Pastures Program of CIAT implies the use of plant and fertilizer material that are adapted to the soil chemical constraints, such as acidity and low soil fertility. Commercially available fertilizer inputs often are inappropriate due to economic or agronomic considerations. In the case of phosphorus, the program seeks to exploit the availability of large rock phosphate deposits throughout Latin America. In Colombia many potassium feldspar deposits have been identified and the preliminary studies showed that the agronomic effectiveness of potassium derived from feldspar can replace potassium applied in chloride form. In the present study, KCl, K feldspar and a mixture of both were evaluated for their effect on the growth of tropical forage plants and the interaction with VA mycorrhizal inoculation.

Methods and Materials

The experiment was conducted in greenhouse in an acid, unfertile and unsterilized Oxisol from Carimagua, Meta, Colombia. The soil was fertilized with (kg/ha): 100, Ca; 10, P; 20, S; 24, Mg and 50, N. The treatments applied were: Zero K; 30 kg K/ha as KCl; 30 kg K/ha as K Feldspar and 30 kg K/ha as a mixture of both. One half of the treatments were inoculated with a mixture of three VA mycorrhizal fungi. The test plants used were: Stylosanthes capitata, Centrosema macrocarpum, Zornia glabra, Andropogon gayanus and Panicum maximum. The plants were sown as monoculture (4 plants/pot) in pots of 3 kg. For each treatment three replicates were used. After 9 weeks plant tops were harvested and dry weight and mineral content determined.

Results and Discussion

Table 1 and 2 show that dry matter production of all plants (with few exceptions) was increased significantly by mycorrhizal inoculation. However, the extent of increase varied among plants and potassium treatments. Generally, the dry weight of plants, inoculated or not inoculated, given KFs was higher than other K treatments. Non-inoculated control, KCl and Mix Zornia, Centrosema Stylosanthes and Andropogon plants did not show any difference among them. This may be due to poor growth of plants caused by less availability of P. Exceptionally good growth of non-inoculated Zornia, Centrosema and Panicum plants given KFs indicates that KFs may have some positive secondary effects on the growth of these plants. This can also be seen if we consider the main effect of fertilizers irrespective of mycorrhizal inoculation (Table 3). Dry weight of Zornia, Centrosema and Panicum was significantly increased by KFS when compared with KCl, with Andropogon and Stylosanthes no significant difference between these sources was observed.

The data clearly indicate that the application of potassium in chloride form can be replaced by potassium feldspar rocks. Feldspars being less susceptible to leaching than the traditional, high soluble fertilizers would release their nutrients slowly over a long period of time, as does rock phosphate. This would also require less frequent appplications and thus reduce the cost. From these results it can also be concluded that there exists a high interaction between mycorrhizal activity and fertilizer use on plant growth.

Table 1. Shoot dry weight (g/pot) of three tropical forage legumes supplied with two sources of potassium and either inoculated (+I) or not inoculated (-I) with mycorrhizal fungi.

Potassium treatments* (kg/ha)	Z. glabra		C. macrocarpum		S. capitata	
	-I	+I	-I	+I	-I	+I
0	3.30	8.20	5.01	8.21	2.25	5.58
30 (KCl)	2.79	9.37	5.05	9.55	2.48	8.17
30 (Mix)	4.40	7.60	6.55	10.60	2.41	5.14
30 (KFs)	8.87	10.31	9.55	10.89	3.41	5.20
LSD at 5%	1.89		2.23		1.55	
Main effect of inoc.	4.84	8.87	6.54	9.81	2.64	5.58
LSD at 1%	1.36		1.54		1.07	

*0, Zero potassium; 30 (KCl), as KCl; 30 (Mix), KCl and K feldspars (1:1); 30 (KFs), as K feldspars.

Table 2. Shoot dry weight (g/pot) of two tropical forage grasses supplied with two sources of potassium and either inoculated (+I) or not inoculated (-I) with mycorrhizal fungi.

Potassium treatments* (kg/ha)	A. gayanus		P. maximum	
	-I	+I	-I	+I
0	3.97	6.46	6.36	7.82
30 (KCl)	5.08	9.38	9.43	11.53
30 (Mix)	5.49	8.72	10.32	15.18
30 (KFs)	5.52	9.37	13.22	16.20
LSD at 5%	1.78		3.26	
Main effect of inoc.	5.02	8.48	9.83	12.68
LSD at 1%	1.22		2.24	

* Same as in Table 1.

Table 3. Main effect of potassium fertilizers on the dry weight (g/pot) of five forage plants grown in unsterilized Oxisol as monocultures.

Species	Potassium treatments*				LSD at 5%
	0	30(KCl)	30(Mix)	30(KFs)	
Z. glabra	5.75	6.08	6.00	9.59	1.40
C. macrocarpum	6.61	7.30	8.57	10.22	1.58
S. capitata	3.02	5.33	3.78	4.31	1.10
A. gayanus	5.22	7.23	7.11	7.45	1.26
P. maximum	7.09	10.48	12.75	14.71	2.30

* Same as in Table 1.

FIELD RESPONSE OF CHILLI TO VA MYCORRHIZA IN BLACK CLAYEY SOIL

By

K.R.Sreeramulu and D.J. Bagyaraj

Keywords--Capsicum annuum, Glomus fasciculatum, P fertilizer

Introduction

Field inoculation trials have shown that growth responses with selected mycorrhizal endophytes can vary from nothing to threefold(Ruehle and Marx, 1979). The main handicap in VA mycorrhizal research has been the production of inoculum on large scale. Hence at present and till suitable methods are developed to mass multiply the fungus the best way to utilize VA mycorrhizal fungi would be to concentrate on nursery raised crop plants which could be easily inoculated with selected mycorrhizal fungi and then transplanted to the field. In an earlier study we reported that isolate I_4 (Glomus albidum) was best in improving growth and nutrition of chilli and that inoculation with G.albidum would result in 50% saving of P fertilizer application in red sandy loam soil (Bagyaraj and Sreeramulu, 1982). The present investigation was carried out to determine whether G. albidum which was the best mycorrhizal fungus for chilli in red sandy loam soil also performs best in black clayey soil.

Methods and Materials

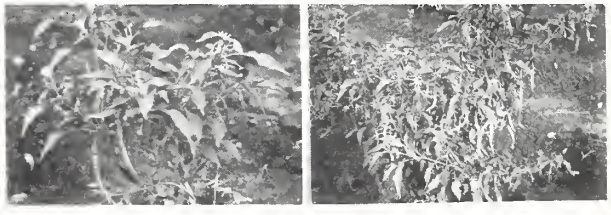
Raised nursery beds(92x30x10cm) were inoculated with 4 different mycorrhizal fungi viz. Glomus fasciculatum(Gf), G.albidum(Ga), G.macrocarpum(Gm) and isolate I_{14} (I_{14}). Chilli seedlings raised in these and control(C) beds were transplanted to the experimental plots 3 m x 1.35 m . There were five inoculation treatments (no inoculation, inoculation with 4 different fungi) at 2 levels of phosphorus (0 P and half P i.e. 37.5 kg P/ha) amounting to 10 treatments. The 11th treatment was full P (75 kg P/ha) with no inoculation. The experiment was laid out in a randomized block design with 4 replications. Plant height, number of flowers, dry weight of shoot and its P and Zn contents, yield of green chillies(total of 4 pickings) and mycorrhizal root colonization were determined 80 days after transplanting.

Results and Discussion

Chilli plants responded well to mycorrhizal inoculation under field condition (Fig.1). The extent of response varied with the isolate (Table 1). Of the 4 fungi studied inoculation with G. fasciculatum caused maximum increase in plant height, shoot dry weight, shoot P and Zn contents, yield and percentage mycorrhizal colonization of the root system. Yield of plants inoculated with G.fasciculatum at half P was more than the uninoculated plants receiving full P suggesting that application of phosphatic fertilizer could be reduced through inoculation with an efficient strain of mycorrhizal fungus. A similar trial conducted earlier in red sandy loam soil showed G.albidum to be the most efficient mycorrhizal fungus. This brings out that the most efficient strain of mycorrhizal fungus in a particular soil type causing maximum symbiotic response need not be the best fungus for the same crop in another soil.

Table 1. Effect of mycorrhizal inoculation and phosphorus on yield of chilli (kg/plot)

Inoculation	P Amendment (kg/ha) 0	37.5	75	LSD at P=0.05
C	0.27	0.37	0.43	
G_f	0.40	0.52		
G_a	0.38	0.41		Inoculation = 0.057 Phosphorus =0.036
Gm	0.32	0.40		
I_{14}	0.37	0.42		



Zero P | Zero P + G.fasciculatum

Figure 1. Effect of G.fasciculatum inoculation on chilli given zero P.

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AN IMPROVEMENT OF "WET-SIEVING AND DECANTING" TECHNIQUE FOR ENDOGONACEOUS SPORES EXTRACTION

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Keywords:filtering apparatus,metallic sieves, nylon filters.

Introduction

The separation of spores and microsporocarps from the soil is a fundamental process in the study of Endogonaceous fungi.
The most wide spread technique of extraction is that of "wet-sieving and decanting" proposed by Gerdmann and Nicolson(1963). However,the removal of the spores from the metallic sieves poses several problems,as does their observation in Petri dishes.

Methods and Materials

We have experimented a modification of the filtering apparatus using nylon filters with standard meshes of the kind used in palynology or in cellular cultures for the separation of protoplasts.
The filters once filled have to be put directly under a stereoscope on a transparent plexiglass grid-lined sheet.
The steps are represented in fig.1.

Results and Discussion

The spores are supported by the filter meshes and they can be counted exactly and manipulated easily.

In a series of 213 extractions in which the same sample was examined filtering 100 gr.d.w. of soil through metallic sieves and an equal quantity through nylon filters of similar meshes (400,250,100 and 40 um) a significant advantage was found in favour of the proposed method ($c=0.294717 \int_{\infty} =0.617$).
This means that a part of the spores is lost when utilizing fixed metallic filters.

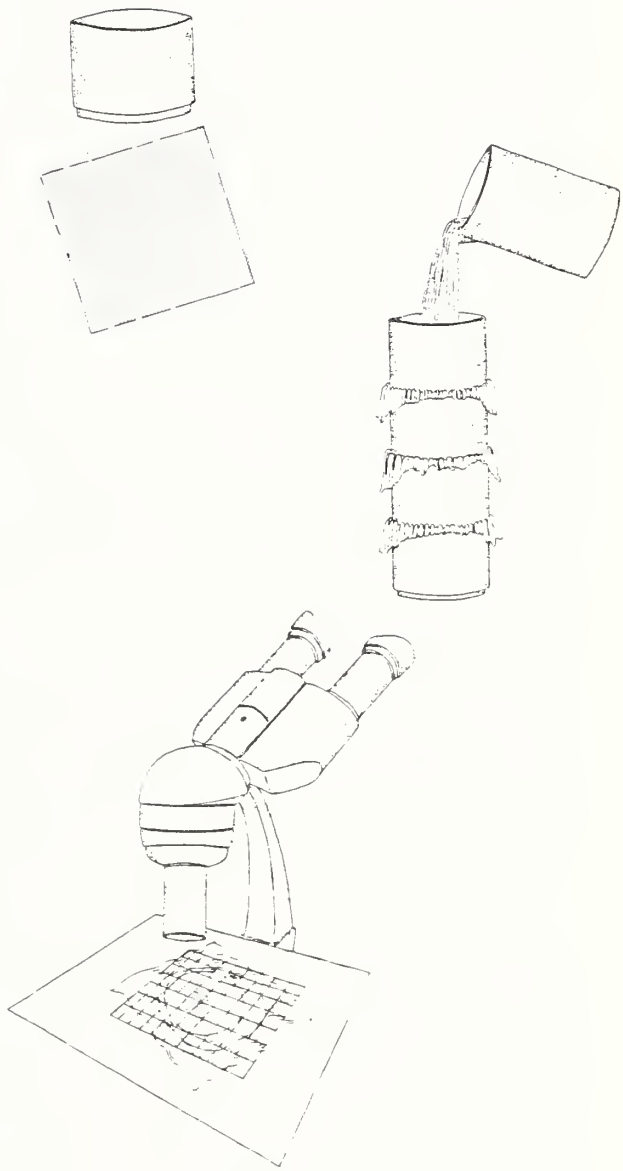

Figure 1.Steps of modified wet-sieving technique using nylon filters.

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EFFECTS OF DIFFERENT VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI ON GROWTH OF *FRAXINUS AMERICANA* CULTIVATED UNDER FIELD CONDITIONS

V. Furlan[1], J.A. Fortin[2] and J.P. Campagna[3]

A field experiment was done to determine the effects of three vesicular-arbuscular mycorrhizal (VAM) fungi on the growth of *Fraxinus americana* in our north temperate climate. Also, we evaluated the possibility of using VAM fungi in the large scale production of white ash seedlings for reforestation.

The experiment was conducted in a low fertility sandy loam soil. It had a pH of 4.8, 2.5% organic matter content, 0.17% N, 69 µg/g P (Bray II analysis), 66 µg/g K, 516 µg/g Ca and 39 µg/g Mg. Thirty-two plots of 2.75 m x 2 m were established and then fumigated with methyl bromide. We had four treatments; three with VAM fungi (*Glomus versiforme*, *Glomus intraradices* and *Glomus monosporum*) and a control, all repeated 8 times. Forty 8-cm white ash seedlings were transplanted in each plot. No fertilizer was applied the first summer (1982), but the following summer (1983) 16 out of the 32 plots received a mixture of N, P, K, and Mg which was incorporated between plant rows at the rate of 100, 50, 120, and 60 kg/ha respectively.

Three months after transplantation plant height of half of the plots was measured (Table 1). The following summer a rapid growth enhancement was observed particularly in plots inoculated with *G. versiforme* and *G. intraradices*. Plants inoculated with *G. monosporum* were much smaller. Control plants remained small and showed some signs of chlorosis. Figure 1 shows the difference between control and inoculated plants. Beginning October 1983, 15 plants from each plot were harvested. Stems and leaves were air dried at 70°C for 5 days, then dry mass was recorded separately (Table 2). The rest of the plants from each plot were divided in 2 lots and used for reforestation at 2 separate sites. The development of the plants will be followed for at least 3 years.

We analyzed the variance of the average dry mass per plant, stems and leaves separately and total of both. The different treatments were compared by orthogonal contrast. This permitted us to observe a very significant difference ($P<0.0001$) between the following treatment: a) Control vs the three VA mycorrhizal fungi; b) *G. monosporum* vs *G. versiforme* and *G. intraradices*.

We did not detect any significant difference between fertilized and non fertilized treatments.

This experiment showed that white ash responded well to at least two VAM fungi; *G. versiforme*, from Oregon, and *G. intraradices*, from Quebec. *Glomus monosporum*, from California, was less efficient probably due to our severe winter conditions since it proved to be as efficient as the other two VAM fungi in a controlled environment experiment.

By using efficient VAM fungi on white ash at transplantation time under field conditions, it is possible to obtain, within one or two growing seasons, plants of a sufficient height for reforestation. Large additions of fertilizer were unnecessary.

Studies on a pilot scale of VA mycorrhized white ash plants for reforestation is presently under way in a nursery.

TABLE 1. HEIGHT (CM) OF WHITE ASH PLANTS THREE MONTHS AFTER TRANSPLANTATION OF 8-CM SEEDLINGS (AVERAGE OF 4 PLOTS/ TREATMENT)

GLOMUS VERSIFORME	23.02
GLOMUS INTRARADICES	21.18
GLOMUS MONOSPORUM	18.41
CONTROL	9.41

TABLE 2. EFFECTS OF DIFFERENT VA MYCORRHIZAL FUNGI ON THE DRY MASS OF *FRAXINUS AMERICANA* CULTIVATED UNDER FIELD CONDITIONS WITH (+) AND WITHOUT (0) FERTILIZATION MEANS OF 8 REPLICATES.

TREATMENT	FERTILIZATION	DRY MASS (G) PER PLANT		
		LEAVES	STEMS	TOTAL
GLOMUS VERSIFORME	+	42.09	75.55	117.64
	0	36.90	71.64	108.54
GLOMUS INTRARADICES	+	38.01	69.75	107.76
	0	36.60	70.68	107.28
GLOMUS MONOSPORUM	+	11.63	15.38	27.01
	0	6.83	9.03	15.86
CONTROL	+	1.25	1.57	2.82
	0	3.60	4.34	7.94
	S.E.	±3.491	±6.157	±9.607

Figure 1. Plants of *Fraxinus americana* in second growing season (net growth time: 7 months). N-M: non-mycorrhizal, M: mycorrhizal.

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COMPARISON OF INOCULA APPLIED TO VA MYCORRHIZAL AND CONTROL PLANTS

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Keywords--VAM inoculum, microorganisms, microbial activity, CO_2 evolution

Introduction

In experiments conducted with vesicular-arbuscular mycorrhizal (VAM) fungi, emphasis has been placed on the establishment of appropriate non-mycorrhizal controls (Gerdemann, 1968, Menge and Timmer, and Linderman and Hendrix *in* Schenck, 1982). Microorganisms associated with the VAM inoculum may affect plant growth and should be introduced into all treatments. Manjunath and Bagyaraj (1981) did not observe an effect of inoculum washings on plant growth but they did not establish if the same organisms were present in all treatments. We conducted two experiments to determine methods for establishing appropriate controls for spore- and pot culture-inoculated VAM plants.

Materials and Methods

Fifty ml erlenmeyer flasks each containing 20 g of soil were autoclaved and then inoculated (experiment 1) at 7 replications per treatment with the following; 1) uninoculated control, 2) 10 surface-sterilized spores of an undescribed *Glomus* sp., 3) 10 non-sterile spores, 4) 10 squashed, non-sterile spores, and 5) spore washings. Experiment 2 had 5 replications per treatment of; 1) pot culture inoculum of the *Glomus* sp., 2) pot culture inoculum plus a wash prepared from the pot inoculum, 3) autoclaved pot culture inoculum, 4) autoclaved pot inoculum plus inoculum wash, 5) pot culture material from an identically treated non-mycorrhizal plant, 6) inoculum wash, and 7) uninoculated control. After inoculation the previously moistened soil in each flask was brought to 16% (w/w) moisture content. Flasks were closed with a loose fitting foam plug and sealed, individually, in an airtight jar together with a vial containing 2 ml of 1M NaOH. The NaOH was used to trap CO_2 evolving from the soil. CO_2 evolution served as an indicator of microbial activity. Each day vials were replaced with clean vials of fresh NaOH solution. The NaOH was titrated with HCl to determine CO_2 absorption. Each experiment lasted approximately two weeks, after which time dilution plating of soil samples was performed to determine colony forming units (CFU) of protein-, chitin-, and cellulose-decomposing organisms, fluorescent *Pseudomonas* spp., and 'total' fungi and bacteria. Protoza were estimated by the most probable number technique. Nematodes were not present in the inocula. Statistical analyses were based on a T-test LSD at $P < 0.05$. Microbial count data were transformed to $\log_{10}$ CFU g^{-1} of soil prior to statistical analysis.

Results and Discussion

In experiment 1, CO_2 evolution was significantly higher for the spore wash and squashed spore treatments than for the washed, live spore treatment. Although significantly less than the other treatments, CO_2 evolution from the surface sterilized spore-inoculated soil was detected. No aerobically culturable microorganisms were obtained from the uninoculated or surface sterilized spore treated soils. Within the live non-sterile spore and squashed spore treatments large variability occurred in the numbers and species of microflora representing the various microbial groups. Replications within the spore wash treatment were much less variable in microbial count data. Chitin-decomposing actinomycetes predominated in the live spore and squashed spore treatments while chitin-decomposing bacteria, fluorescent *Pseudomonas* spp., and protein-decomposing bacteria predominated in the spore wash inoculated soils.

In experiment 2, all of the various types of control inocula produced significantly less CO_2 than the VAM pot culture inoculum. VAM fungal spore germination and hyphal growth may have contributed to this difference and not the activity of the non-mycorrhizal soil microflora. The sterile controls remained sterile. Only the autoclaved VAM pot culture inoculum plus inoculum wash treatment was not significantly different in all microbial group counts from those of the non-sterile VAM pot inoculum treatment. The cellulose-decomposer population was significantly lower in soils receiving the inoculum wash than any other treatment. The organic matter content in the various treatments may have influenced the number of cellulose-decomposers found. In the non-mycorrhizal pot culture treated soils, only total fungal and total bacterial colony counts were not significantly different from those of the VAM pot culture treatment.

Conclusions

CO_2 evolution from the various treatments indicated that there were significant differences in microbial activities between the VAM and control inocula. When inoculating with VAM fungal spores, we recommend the addition of an inoculum wash to all treatments. In studies where VAM pot culture inoculum is used, the most appropriate control is one receiving sterilized VAM pot inoculum plus a wash prepared from the unsterile VAM inoculum. Inoculum from a non-mycorrhizal plant is not recommended for the control.

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INFLUENCE OF MYCORRHIZAL FUNGI AND RHIZOBIUM INOCULATION ON CHICKPEA AND GROWTH CHARACTERISTICS OF LUMEN BACTERIA

By

A. K. Varma, K. Singh and H. D. Peck, Jr.

Keywords -- Glomus, Gigaspora, Rhizobium (Rhiz.), Pyrophosphate, Vesicular Arbuscular mycorrhiza (VAM), Lumen bacteria

Introduction

Spores of Glomus macrocarpum, G. geosporum, G. fasiculatum, Sclerocystis rubiformis, Gigaspora gigantea, Gi. gilmorei, Gi. coralloidea and Gi. calospora were isolated from the rhizospheres of xerophytic plants growing in semi arid zones around Northern India.[1] The effect of VAM together with effective Rhizobium on a tropical legume, Chickpea (Cicer arietinum) was studied. Several strains of rod and coccoid anaerobic bacteria were isolated from the lumen of Glomus and Gigaspora. A possible physiological significance of these inhabiting bacteria has been attempted.

Methods and Materials

Plant growth experiments were conducted in earthern-ware pots filled with sterile soil. Trace element analysis of the root segments was done by micro-probe X-ray analysis and ATP by luciferin-luciferase procedures. Hungate technique was employed to screen and study the lumen bacteria.[2] Cell counts were done using counting chamber.

Results and Discussion

"Dual inoculation" had an overall beneficial effect on the plant growth, root nodulation (Fig. 1), grain yield, leghaemeglobin and ATP contents (Table 1). Total calcium, potassium, phosphorus and sulfur nutrients uptake were enhanced in the root system receiving the VAM and Rhizobium inoculation (Table 2).

Table 1. Effect of dual inoculation on chickpea

Treatment	grain yield per plant(g)	root nodules fr. wt./ plant(g)	leg Hb. mg/g fr.wt. nodules
Rhiz.	7.08	5.96	3.26
VAM	7.94	1.22	0.29
Rhiz. + VAM	9.48	8.38	8.02
C.D. at 5% level	0.797	1.03	(average of 12 plants)

Table 2. X-Ray analysis of elements in roots

Treatments	Ca	K%	P(ppm)	S(ppm)
Rhiz.	2.16	0.88	1301	703
VAM	2.03	1.88	2030	1017
Rhiz. + VAM	4.27	1.65	2504	1490

The bacterial isolates (Fig. 2) from lumen of endomycorrhizal spores were able to utilize inorganic pyrophosphate as a source of energy for growth (Table 3), a phenomenon representing the simplest ATP generating system in the biological world.[3,4] Growth as determined by cell number is proportional to the concentration of pyrophosphate over a limited range and the growth is accompanied by the hydrolysis to orthophosphate. Pyrophosphate is formed by biological system and is reported to accumulate as polyphosphate granules in several fungi and bacteria.[5,6]

Table 3. Growth of lumen bacteria on minimal basal medium (0.1% Difco yeast extract)

Isolates	Cell number/ml volume minus PPi	plus PPi
Rod forms	5.98×10^4	2.7×10^6
Coccoid forms	3.71×10^3	6.43×10^4

Mycorrhizal fungi, nitrogen fixing Rhizobium and energy liberating pyrophosphate utilizing lumen bacteria seem to exhibit potentials for beneficial impact on tropical legume with reference to its yield, productivity of food, feed and protein.

LUMEN BACTERIA ROOT NODULATION IN CHICKPEA

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CRYOPRESERVATION OF GENOTYPES OF VA MYCORRHIZAL FUNGI

By I.C. Tommerup and K.B. Bett

Keywords--Glomus fasciculatum, osmotic stress, dehydration tolerance, propagules, survival, mycorrhizal segments

Introduction

Preservation of living fungi by cryogenic methods has many major advantages. Fungi have inherent genetic instability and preservation eliminates degeneration and mutation. The long-term conservation of an isolate provides insurance against loss, contamination and genetic change and a more practical means of maintaining cultures. Some VAM fungi do not readily spore in natural habitats or pot culture. Therefore the aim was to develop, using low temperature techniques, a scheme for the long-term preservation of VA mycorrhizal colonies in roots.

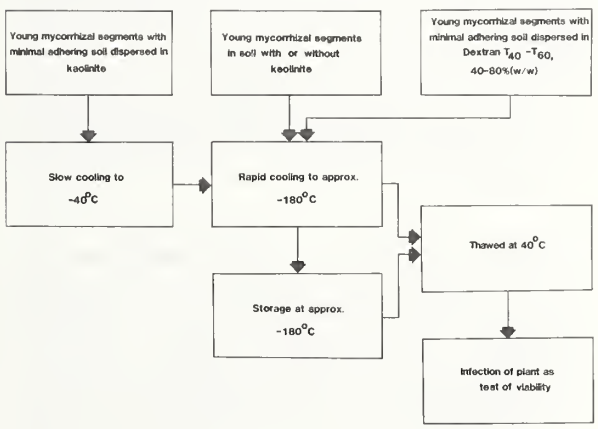
Methods

Cryopreservation involves freezing and storage at low temperatures. Cryoprotective compounds are water miscible substances which can alleviate problems associated with ice crystallization. Compounds which penetrate cell membranes and large molecular weight non-penetrating compounds were used. Glomus fasciculatum in mycorrhizal segments has several anatomical structures and the capacity of these to act as propagules was examined.

Results and Discussion

A scheme for freezing, in liquid nitrogen, young mycorrhizal segments without spores has been developed, and their long term survival is being tested.

A SCHEME FOR THE CRYOPRESERVATION OF VAM FUNGI.



Survival of G. fasciculatum depends on the cryoprotectant and the freezing rate (Tables 1,2). Some cryoprotectants at the concentrations used for other microorganisms or cell suspensions can have adverse effects on the survival of unfrozen G. fasciculatum. The compounds involved penetrate the plasma membranes of VAM fungi and the host plants, and the effect appears to be due to problems of osmoregulation. The problems should be eliminated by modifications in techniques and these aspects are currently being examined. Solution of this problem will enable the value as cryoprotectants of these penetrating compounds to be tested.

Table 1. Effect of cooling rate on survival of G. fasciculatum.

Young mycorrhizal segments in:	Survival (% of control at 20°) Fast to -180°	Slow to -40° + Fast -40 to -196°
Soil (undisturbed core) (1.5g)	0	75
Soil (1.0g) + kaolinite (0.2g)	67	72
Kaolinite (0.5g)	0	46
Dextran $\bar{M}w$ 40,000 (44% (w/w))	2	2
Minimum adhering soil	0	0

Table 2. Effect on survival of storing G. fasciculatum in solutions of penetrating compounds for 1 hour at 20°C before freezing.

Solution	Survival (% untreated control) 20°	Fast or slow freezing to -180°C
Deionised water	100	0
Glycerol (15% w/v)	4	0
Skim milk + glycerol 4.25% + 10% (w/v)	50	0
8.5% + 10% (w/v)	13	0
Dimethylsulphoxide 3.0% (w/v)	130	0
Dimethylsulphoxide + Dextran $\bar{M}w$ 40,000 3.0% (w/v) + 44% (w/w)	80	0

Three components of mycorrhizal segments are able to regrow, grow through the soil and establish a colony in a host root (Table 3).

Table 3. Time taken by anatomical structures of G. fasciculatum in root segments to regrow and initiate colonies in host roots.

Structure	Unfrozen		Frozen	
	Regrowth from cut ends	Initiate colony	Regrowth from cut ends	Initiate colony
Intraradicle hyphae	1d	2d	7-10d	14-21d
Extraradicle hyphae attached to entry structure	1-2d	2-3d	9-14d	14-21d
Detached extraradicle hyphae	2-5d	10-14d	14-21d	14-28d

INTERACTING EFFECTS OF APPLIED P, LIME, AND VAM ON SOYBEAN

By

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Keywords--Glycine max, var. Essex, elemental content, seed yield, chloride, Glomus

Introduction

A limited response to lime has been obtained with soybeans at high rates of P as $Ca(H_2PO_4)_2$ during the last 3 years in field studies on an acid (pH 5.0), infertile Wynnville sil in Lawrence County, AL. Lack of response to lime on such an acid soil might be attributed to partial alleviation of the adverse effects of soil acidity with high rates of $Ca(H_2PO_4)_2$. Also, high P rates might render the native mycorrhiza ineffective in stimulating plant growth. Thus, a follow-up greenhouse study was conducted to evaluate plant-growth-stimulating factors associated with two VAM inocula as influenced by lime and fertilizer P.

Methods and Materials

Soil pH levels of 5.0, 7.0, and 8.0 were achieved by adding 0, 1.5, and 3.0 g/kg of 4:1 $Ca:MgCO_3$ to a Wynnville surface soil (pH=5.0, CEC=4.3, P=4 ppm). After a 2-week lime-soil equilibration, 0, 20, 40, 80, and 160 mg/kg of P as KH_2PO_4 was applied with KCl (403 mg/kg total K) and 50 mg/kg of micronutrients. Three control treatments at each lime level were tested for fertilizer influences without VAM inocula: no fertilizer; 320 mg P/kg as KH_2PO_4 + micronutrients; and 320 mg P/kg as $Ca(H_2PO_4)_2$ + micronutrients + 403 mg K/kg as KCl. Glomus fasciculatus Gerdemann or Glomus fasciculatus from Natchez, Mississippi was added in a layer 3-4 cm below the soil surface as a mixed propagule inoculant (20 g/pot) into 6 kg of soil per pot. Essex soybeans were Rhizobia inoculated (3 plants/pot) and watered daily to 18% w/w moisture. Plant height and dry matter of seeds, pods, roots, and stems were taken at maturity. Seeds were analyzed for N, P, K, Ca, Mg, and Cl. Roots were examined for colonization and spores were estimated.

Results and Discussion

Phosphorus accounted for 74% of the influence in the P x lime x VAM interaction for increasing seed yield (Table 1). Both VAM inoculants enhanced seed yield similarly (+12%) at all P levels in the 0 and low lime treatment. The Gerdemann isolate was better (+20%) than the Natchez (+3%) in the high P and high lime. The Natchez isolate was better than Gerdemann with the no P and high lime treatment (+272% vs. 0). VAM, on the average, increased pod weight (+12%) and seed Cl (+35%) and reduced plant height (4%), seed content of N (8%), P (12%), K (3%), Ca (15%), and Mg (5%). Seed Cl was positively correlated to the applied KCl and was reduced by both lime and P. Fertilizer comparisons made with $Ca(H_2PO_4)_2$ (320 mg P/kg), and KH_2PO_4 indicated an average reduction of 45% in seed yield when KCl was applied as the only source of K. Chloride uptake was increased by both VAM and P. Soybean varieties tolerant to Cl contain less than 173 ppm Cl in seeds (Parker et al., 1983). This level was equaled or exceeded except in the highest KH_2PO_4 level or when KCl was omitted. These results are in agreement with those obtained with wheat and barley (Buwalda et al., 1983). However, the Natchez isolate interacted with the high lime level to reduce seed Cl 33% to near the tolerant threshold. VAM enhancement of anion uptake may be reducing root carboxylate synthesis (Israel and Jackson, 1982) and/or interferes with the vitality of the K-malate "pump." Salt-tolerant soybean varieties do not translocate appreciable amounts of Cl^- to the shoot but accumulate Cl^- in roots (Läuchli and Wienke, 1979). The Gerdemann isolate colonized a higher percentage of the roots than did Natchez (75% vs. 50%) and was more intensely colonized. The mechanism of the VAM-KCl influence on plant growth needs further investigation.

Table 1. Influence of lime, P, and VAM on soybean seed yield (g/pot).

Lime (g/kg)	P (mg/kg)	VAM[1/] Gerdmann	Natchez	None
0	0	0.2a	0.7a	0.0
	20	11.0	10.0	6.1
	40	16.2	15.1	13.2
	80	26.7	23.4	23.7
	160	41.6	45.2	40.8
1.5	0	7.2	5.8	1.9
	20	15.1	18.4	12.8
	40	20.9	22.9	19.3
	80	30.6	31.1	23.1
	160	51.3bc	55.0ab	43.4c
3.0	0	3.7b	14.9a	4.0b
	20	26.7	26.8	23.8
	40	36.5	34.5	31.5
	80	39.1ab	45.7a	34.3b
	160	60.5a	52.1b	50.4b

[1/] Means followed by the same lettering within a row are not different (P=0.05) by DNMRT.

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THE SYMBIOSIS Rhizobium-Glomus IN Leucaena leucocephala.

By

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Keywords: Leucaena, Glomus, Rhizobium, phosphate rock, ordinary superphosphate.

Introduction

Leucaena leucocephala is a promissory plant for warm regions of the world due to its high protein and biomass yields. The purposes of this work were: (a) to study the effects of the induced symbiosis Rhizobium + Vesicular-arbuscular endomycorrhiza (VAM) in Leucaena when it is established in marginal soils and (b) to test the hypothesis that double inoculation can substitute the nitrogen fertilization and diminish the phosphorus application requirements for a high biomass production. Verification of such hypothesis was performed by means of a greenhouse experiment where the effects of the double symbiosis were analyzed under four different levels of P_2O_5 applied with two P sources: ordinary superphosphate (OSP) and phosphate rock (PR).

Materials and methods

Two Leucaena seedlings cv. Peruana were placed in pots with 4 kg of soil (pH = 5.3 and 6.2 ppm of P Bray-1) previously fumigated with methyl bromide. A factorial experiment (3X2X2X4) was established with the following four factors: nitrogen (0 ppm N, 10 ppm N and inoculation with Rhizobium), mycorrhiza (with or without VAM), P source (OSP and PR) and P levels (0, 50, 100 and 150 ppm of P_2O_5). The combination of factors and levels yielded 48 treatments including the control. Plant receiving Rhizobium treatment were inoculated with nearly 1.8×10^9 rhizobia (strain CIAT 1920). To induce mycorrhizal infection one gram of maize root (80% of infection) + 50 g of soil with spores of a Glomus sp. strain from our collection were used as inoculant. Both microsymbionts were previously characterized as effective in Leucaena (1). Three replications of each treatment were run. The crop was harvested 130 days after transplant.

Results and discussion

Increases in the weight of the dry nodules, dry root weight, plant height, dry weight of tops and total plant biomass were observed with the increment of the P_2O_5 level. Maximum values of the above mentioned variable, with exception of plant height, were obtained in the treatment double inoculation + 150 ppm of P_2O_5 (OSP). Inoculating only with VAM the value of these variables were the same as those obtained with the addition of 150 ppm of P_2O_5. In spite of negative interaction VAM x P rate, it was observed that the dry weight of the root and the total biomass tended to increase when the level of P applied was increased (Table 1 and Fig. 1). The effect of mycorrhiza inoculation in the absence of P_2O_5 was smaller than that of mycorrhiza inoculation plus 100 or 150 ppm of P_2O_5 (OSP), although statistically there were no differences. These results can be explained by the decrease in the mycorrhizal infection (Fig. 1) with the increase of the P rate. The latter means a greater availability of this element in the soil and a plant was less dependent on the fungi, whose function was partially substituted by an initially well developed root systems. The results indicate that under these experimental conditions, which are comparable to those prevailing in a propagation nursery, the best option for Leucaena leucocephala growth is the restitution of the mycotrophic condition and the symbiosis with Rhizobium.

Table 1. L. leucocephala growth with different treatments[1/]

Treatment[2/]	Plant height	Dry weight. Tops	Roots
Rh.+VAM+ 150 ppm (OSP)	40.9ab	12.3a	12.0a
VAM+OSP (150 ppm)	37.3abcde	10.8ab	12.0a
VAM+PR (150 ppm)	41.9a	12.3a	11.1abc
150 ppm P_2O_5 (OSP)	37.9abcd	10.5abc	11.1abc
150 ppm P_2O_5 (PR)	30.0de	12.3bc	10.1abcd
VAM inoculated	39.6abc	10.4abc	8.7cd
Rhizobium sp.	8.1f	0.6d	0.5e
Control	8.8f	0.7d	0.5e

1/Means with the same letter within each column are not statistically different with the Duncan multiple range test (P=0.05).

2/Rh.=Rhizobium, VAM=Glomus sp., OSP=ordinary superphosphate and PR=phosphate rock.

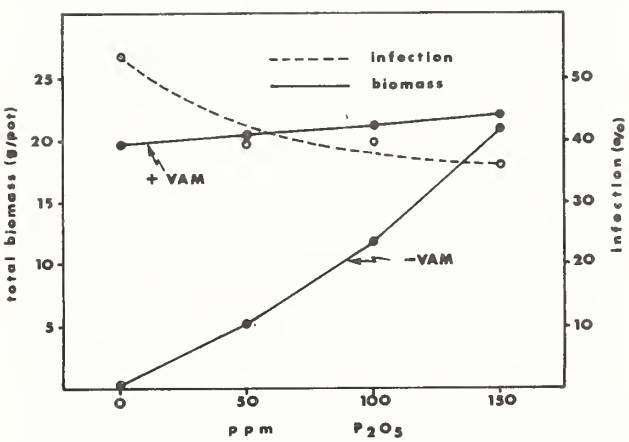

Figure 1. VAM x level of P_2O_5 interaction on the biomass production and % of infection in L. leucocephala.

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Proyecto FAO/159 AGPD-RE CP-000001

Dedicado al Dr. Oscar Brauer Herrera. FAO-Roma.

THE V-A ENDOMYGORRHIZA AND ITS EFFECT OF THE DEVELOPMENT OF THREE ARBOREUS LEGUMES.

By

Ferrera-Cerrato, R. and Villerías, S.J.

GEDAF-GP CHAPINGO, MEXICO

Keywords: Acacia cyanophylla, Eysenhardtia polystachya, Piscidia communis, Rhizobium sp. Glomus sp., double inoculation.

Introduction.

Absorption of nitrogen and phosphorus, vital elements for legume development, is influenced by symbiotic microorganisms such as Rhizobium and Glomus. The purpose of this work was to test the effect of the association of Rhizobium and Glomus on the growth and development of three arboreus legumes Acacia cyanophylla, Eysenhardtia polystachya and Piscidia communis. For this purpose a greenhouse experiment was established with the three above mentional legumes, inoculating each with Rhizobium (R) strains and the vesicular-arbuscular mycorrhizae (V-A) Glomus sp. (G).

Materials and methods

Twenty-nine days old seedlings were transplanted to black polyethylene bags with 600 g of mixture of P deficient soils, sand and organic matter (2:1:1) desinfected with methyl bromide. The treatments were combination of 4 Rhizobium strains (SLP, CSAT35, CSAT39, and GP44), one Glomus sp. strain from our collection, double inoculation (mycorrhizae and Rhizobium strains) plus a control. The experiment had a total of 10 treatments with 5 replications for each plant species under a completely randomized design.

Results and discussion

The more important results are shown in the table 1 for A. cyanophylla, the double inoculation effect on the height parameter can be observed where the strain R(SLP)+G being the best with a plant height of 26.9 cm versus the control of 15.5 cm. In the dry weight of the plant tops no statistical differences were found, but the high value was for the same strains R(SLP)+G with 1.15g versus the control with 0.75g. The highest nodule, production was with strain R(CSAT35)+G with 115. For E. polystachya, (Table 1), the effects of the treatments were more clear and the best was R(GSAT35)+G for both height and, dry weight of the plant tops (20.4 cm and 0.52 g versus the control with 4.0 cm and 0.05g). It is interesting that plants only inoculated with Rhizobium had a poor development compared to that of plants inoculated with both symbiont. The nodule production was low in this species so the main effects could be attributed to the mycorrhiza. In P. communis (Table 1) the treatment R(CP44)+G had a height of 11.2 cm versus 5.6 cm for treatments R(CSAT35) used as reference; regarding the dry weight of the plant tops, the highest value was for the same treatment (3.67g versus 1.08 g of the control), these results showed statistically significant differences. Regarding quantity of nodules treatments R(CSAT39) and R(GSAT39)+G were the highest with 197.6 and 151.2 respectively, but had no apparent effect on plant development. Again it can be mentioned that diverse factors condition the effectiveness of these symbionts. The response of the mycorrhizal infection in A. cyanophylla and E. polystachya was relatively low, unlike that of P. communis (Table 1). It is known that the highest infections do not always produce the best effects in the development of plants (studies carried out in onion by Ferrera-Gerrato and Macedo, 1981), but that these effects depend of the effectivity of the strains. Also, the responses of mycorrhizal infections is probably limited by the availability of P in the soil (Munns and Mosse, 1980). It is concluded that the utilization of these microorganisms at the nursery level would be of great importance for the good development of these plants.

Table 1. Effect of double inoculation with Rhizobium and Glomus in three arboreus leguminosae*.

Sp/Treat.	Height (cm)	Dry weight of plant top (g)	Total of nodules produced	% of total mycorrhizal infection
A. cyanophylla				
Control	15.5 b	0.75a	5.0 b	0
R(SLP)	18.1ab	0.90a	11.2 b	0
R(SLP) + G	26.9a	1.15a	6.0 b	33
R(CSAT35) + G	22.4ab	0.96a	115.0a	14
Glomus sp.	18.1ab	0.96a	3.6 b	29
E. polystachya				
Control	4.0 c	0.05 b	0 a	0
R(CSAT35)	9.9 bc	0.20 b	0 a	0
R(CSAT39) + G	20.4a	0.52a	1.0a	21
Glomus sp.	15.3 b	0.41ab	0.6a	12
P. communis				
R(CSAT35)	5.6 c	1.08 c	55.6 b	0
R(CP44)	7.0 bc	1.81 c	47.0 b	0
R(CSAT39)	5.4 c	1.35 c	197.6a	0
R(CSAT39) + G	7.6 b	1.89	151.2a	79
R(CP44) + G	11.2a	3.67a	72.0 b	81
Glomus sp.	8.3 b	2.83 b	0.33 e	77

*Means of each column having the same letter are not significantly different. Tukey test (P=0.05) R= Rhizobium, G= Glomus.

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Dedicated to Dr. Carlos Casas Gampillo. GINVESTAV-IPN.

AN ISOLATION TECHNIQUE FOR THE INTRAMATRICAL VESICLES OF VESICULAR-ARBUSCULAR MYCORRHIZAE

S. Jabaji-Hare, S. I. Sridhara and B. Kendrick

Key words: Allium porrum, Glomus intramatrical vesicles, sucrose gradient.

Introduction

The few Biochemical investigations that have been made of VAM fungi have usually involved only their extramatrical spores. We have been able to liberate and purify large numbers of intramatrical vesicles. This may simplify propagation and open up new analytical possibilities.

Materials and Methods

A culture of an unnamed species of Glomus (Herb. DAOM 181602) supplied by Dr. A. Fortin, Université Laval, Ste. Foy, Québec, formed vesicles in the host roots, but no external spores. Inoculum production and multiplication was carried out in roots of leek (Allium porrum L.). After 5 months, the plants were removed from the growth medium and the roots were washed with distilled water to remove soil particles and debris.

Thirty to forty grams of clean, colonized roots were macerated in 60-80 mL of cold 0.3 M sucrose--0.05 M $NaHCO_3$ at pH 7.0 in a mortar and pestle. The root suspension was further homogenized in a Polytron type PT 20ST for 3-5 minutes to ensure that all root tissue was properly comminuted. The homogenate was filtered through a single layer of cheesecloth to remove root tissue. The filtrate was retained and was adjusted to 15 mM CsCl by adding 1 M CsCl, and then centrifuged in a discontinuous sucrose gradient prepared as follows: 8.0 mL of 1.3 M sucrose--15 mM CsCl was placed in the bottom of each of six cellulose nitrate centrifuge tubes, and 30 mL of filtrate was carefully layered on top of the 1.3 M sucrose. The tubes were then centrifuged at 82000 g for 180 min. Then aliquots of the two layers, of the conspicuous interface zone between the layers, and of the pellet at the bottom of the tube, were examined microscopically for the presence of vesicles. Vesicles were found only in the interface zone. This zone was collected by syringe, diluted with five times its volume of 0.05 M $NaHCO_3$ at pH 7.0, and washed twice by centrifugation at 20800 g for 45 min at 4°C. The whole extraction procedure was repeated six times.

After each extraction the sedmineted vesicles were dispersed in 2 mL of 0.05 $NaHCO_3$ and vortexed to ensure uniform distribution of vesicles. One 10.0 uL sample was taken, diluted tenfold, and the vesicles were counted under a microscope.

Results and Discussion

The well defined interface zone between the layers of the gradient contained purified vesicles. The pellet at the bottom of the tube contained only plant cells, while the other layers contained neither vesicles nor plant debris. The procedure appears to be more efficient than other existing methods for purifying spores (Mertz et al., 1979; Kucey and McCready, 1982). Our method yielded an average of 46000 vesicles per operator day, and this yield could be increased if larger quantities of colonized roots were available. Examination of the purified vesicles with the light microscope showed very little evidence of damage. Cells were not broken, had retained their contents, and did not appear plasmolyzed (Fig. 1).

The recovery of vesicles in large quantities from mycorrhizal roots is essential for biochemical studies of VAM fungi such as Glomus intraradices Schenck et Smith (Schenck and Smith, 1982), neither of which forms spores or sporocarps outside the root. Our technique will also allow biochemical comparisons of vesicles and spores in other vesicular-arbuscular mycorrhizal fungi.

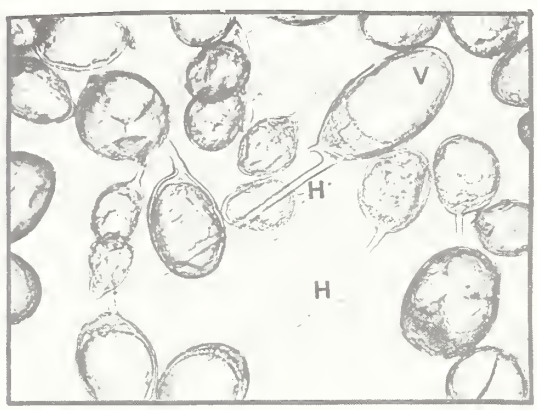

Figure 1. Pure isolated vesicles of Glomus sp.; note attached hyphae and absence of root material. (Nomarski interference Contrast) X208.

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INFLUENCE OF MYCORRHIZAE, PHOSPHORUS, AND LIMING ON BLACK LOCUST GROWTH

By

J. A. Hetrick and E. M. Jencks

Keywords - Robinia pseudoacacia, Glomus, minesoil

Introduction

Revegetation of minesoil may require reduction of soil acidity, addition of N and P fertilizers, and the presence of mycorrhizal fungi. Leguminous plants such as black locust may be efficient minesoil colonizers because of their dual symbiosis with Rhizobium and endomycorrhizal fungi. This study determines the effect of endomycorrhizal fungi on black locust growth in limed and nonlimed, nonsterile, minesoil amended with either rock phosphate (RP) or triple superphosphate (TSP) fertilizer.

Materials and Methods

A sandy loam minesoil formed from the overburden of the Upper Freeport coal seam in Preston County, West Virginia which had a pH of 3.4 and 7.4 ppm double acid extractable P was used as the growth medium. Limed treatments received 7,616 kg/ha of hydrated lime and fertilized treatments received 48.2 kg P/ha of RP or TSP. Mycorrhizal treatments received 230 g/pot of peat:vermiculite containing Glomus mosseae and Glomus etunicatum culture:non-inoculated pots received sterilized peat:vermiculite and inoculum filtrate. Black locust seeds, inoculated with commercial Rhizobium inoculum, were planted into the minesoil. After 6 months growth tree seedling were measured and mycorrhizal root colonization and nodule intensity were assessed.

Results and Discussion

Stem height increased significantly in the limed, mycorrhizal, no P treatment (Table 1) but liming or mycorrhizal inoculation alone were ineffective. Mycorrhizal intensity was not significantly increased by liming in the no P treatment (Table 2) but nodulation was increased in the limed, mycorrhizal, no P treatment.

Stem height increased significantly with the addition of lime in the P fertilized treatments regardless of mycorrhizal inoculation. Stem height increased significantly with the addition of mycorrhizae in the RP, no lime and TSP, limed treatments (Table 1). Mycorrhizal intensity increased significantly in the limed, P fertilized treatments (Table 2) but nodulation was unaffected by mycorrhizal inoculation in the P fertilized, limed treatments.

These results suggest that mycorrhizal inoculation and liming benefit black locust growth even if the minesoil is not fertilized. However, a P fertilization level commonly utilized for successful revegetation did not suppress the mycorrhizal response regardless of P fertilizer solubility. The failure of P fertilizers to reduce the mycorrhizal response suggests either the P fertilization level was not supraoptimal or the minesoil reduced available P because of high soil P fixation capacity. The increase in mycorrhizal intensity in only limed, P fertilized treatments suggests that a minimum soil P level is required for successful root colonization. Alternatively, increased root growth in limed, P fertilized treatments may allow earlier and more successful contact between roots and mycorrhizal inoculum.

Table 1. The influence of mycorrhizae, liming, and phosphorus fertilization on black locust growth.[1/]

	Stem Height (cm)			
Phosphorus	No Lime		Lime	
Treatment	NI*	I	NI	I
No P	4.7^{f}	8.4^{f}	6.4^{f}	15.8^{cd}
RP	6.9^{f}	16.5^{c}	19.5^{b}	24.0^{b}
TSP	9.8^{ef}	13.9^{de}	22.9^{b}	30.3^{a}

* NI-noninoculated; I-inoculated

1/ Numbers followed by the same letters are not significantly different (P=0.05)by Duncans Multiple Range Test.

Table 2. The influence of mycorrhizae, liming, and phosphorus fertilization on mycorrhizal intensity in black locust.[1/]

	Mycorrhizal Intensity Rating [2/]			
Phosphorus	No Lime		Lime	
Treatment	NI*	I	NI	I
No P	0.00^{c}	1.62^{b}	0.05^{c}	1.59^{b}
RP	0.00^{c}	1.71^{b}	1.13^{b}	2.74^{a}
TSP	0.23^{c}	1.18^{b}	0.25^{c}	3.09^{a}

* NI-noninoculated; I-inoculated

1/ Numbers followed by the same letters are not significantly different (P=0.05)by Duncans Multiple Range Test.

2/ Intensity Rating on 1 cm root segments: 1=1-25%; 2=26-50%; 3=51-75%; 4=76-100%.

YIELD RESPONSE OF CASSAVA TO FIELD INOCULATION WITH VA-MYCORRHIZA IN ACIDIC SOILS.

By

E. Sieverding

Keywords Factors influencing response, Glomus manihotis, Glomus occultum, Entrophospora colombiana, P sources

Introduction

It is well known that cassava depends obligately on VA-mycorrhizae for phosphorus (P) nutrition, and that Colombian soils are highly variable in quantity and quality of their indigenous mycorrhizal population (Howeler and Sieverding 1983). Field inoculation trials were conducted to study the dependency of inoculation response on the introduced fungus, the applied P source and the quantity of the indigenous mycorrhizae.

Methods and Materials

Trials were conducted at 6 sites in the mountain region of the Cauca Department, Southern Colombia (1500 m.a.s.l.). The chemical characteristics of soils are given in Table 1. They were fertilized with 0.5 t/ha dolomitic lime, 50 kg N/ha as urea, 50 kg P/ha as Huila rockphosphate (HRP) or triple superphosphate (TSP) and 50 kg K/ha as KCl. Lime and HRP were incorporated, all other fertilizer were applied in side bands to the plants. Cassava cv. CMC-92 was planted in vertical position in 4 replicated treatment plots, each of 22.4 m^2 (35 plants). Inoculum (400 g/plant) was applied under the stake at planting. It consisted in soil/root material from tropical kudzu on which G. manihotis (Howeler, Sieverding et Schenck), E. colombiana (Spain et Schenck) or G. occultum (Walker) had been multiplied. The trials were harvested after one year.

Results and Discussion

On the average of both P sources (Table 2), G. manihotis increased yields by 33%, and the other species to a lower extent. G. manihotis was most effective with TSP, and G. occultum with HRP. E. colombiana (strain C-10) was known not to be well adapted to the low temperature (20°C). Results in Table 3 show clearly that, in absolute terms, highest yield responses to field inoculation occur when the indigenous mycorrhizal population is low. From all soil chemical characteristics the quantity of mycorrhizae is most likely to be correlated with the soil's organic matter content. Without doubt, rapid, easy methods have to be developed to predict the quantity and quality of the indigenous mycorrhizae.

Table 1. Chemical characterization of soil sites

Site	OM %	pH	P 1/ ug/g	Al	Ca	Mg	K
				---- meq / 100 g -----			
A	4.8	4.4	3.6	2.5	0.9	0.3	0.1
B	9.8	4.9	3.8	1.1	1.1	0.2	0.1
C	18.9	5.0	1.4	0.9	2.5	0.7	0.3
D	8.5	4.3	2.9	3.3	1.6	0.9	0.2
E	11.6	5.1	1.3	0.7	0.6	0.2	0.3
F	3.2	5.0	1.2	1.4	1.9	1.2	0.2

1/ Bray II extr. P

Table 2. Effect of P sources and field inoculation on root yield (t/ha) of cassava

Inoculation 1/	Site A		Site B		Average	
	TSP	HRP	TSP	HRP	TSP	HRP
Not inoc.	18.5	11.3	13.1	12.7	15.8	12.0
C-1-1	22.9	17.3	18.1	15.5	20.5	16.4
C-10	19.7	18.7	16.0	11.8	17.9	15.3
C-33-1	21.1	20.4	14.0	14.6	17.6	17.5

1/ Not inoc.:Indigenous mycorrhizae; C-1-1: G. manihotis; C-10:E. colombiana; C-33-1:G. occultum

Table 3. Interaction between indigenous mycorrhizae and field inoculation response (all trials with 50 kg P/ha as HRP)

Site	Infective propagules/ 100 g soil 1/	Root yield (t/ha)		Yield increase (t/ha)
		Not inoc.	Inoc. 2/	
C	1717	17.7	15.7	- 2.0
D	823	24.7	27.8	+ 3.1
E	213	13.5	16.9	+ 3.4
F	103	21.2	27.1	+ 5.9

1/ Defined by MPN test; 2/ Inoculated with mixture of G. manihotis / E. colombiana

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ROCK PHOSPHATE AS A SOURCE OF P FOR OPTIMUM VA MYCORRHIZAL DEVELOPMENT AND GROWTH RESPONSE IN SOILLESS MEDIA

By J. H. Graham and L. W. Timmer

Key words--Peat-based media, P-availability, greenhouse production, citrus rootstocks

Vesicular-arbuscular mycorrhizal (VAM) colonization is reduced and growth responses eliminated when soilless media are fertilized with soluble P. Soilless media have lower P-adsorption capacity and P is more available than in mineral soils (1). However, it is unclear whether components of soilless media, such as peat, directly affect VAM fungi or if the effects are attributable to greater P availability in the medium. Here we examine P release from superphosphate (SP) and rock phosphate (RP) in peat-perlite medium in relation to VAM colonization and growth of a citrus rootstock, Carrizo citrange (CC).

Materials and Methods

1) Two P-sources: Florida RP (200 mesh, 15% P), and ordinary SP (40 mesh, 8.6% P).
2) Four rates of each P source: RP - 0.75, 1.5, 3.0, 7.5 mg P/cm^3 medium, SP - 0.025, 0.05, 0.1, 0.25.
3) Three-mo-old CC inoculated with Glomus intraradices Schenck & Smith and uninoculated.

Results and Discussion

Loss of P from SP-amended medium was exponential, whereas in RP was linear (Fig. 1). If the curves are extrapolated to zero time, over 80% of the applied P from SP was leached by 9 weeks compared to 25% from RP.

VAM colonization of CC was significantly lower in SP-amended media than in RP (Fig. 2). After several weeks, P from SP was leached to low, apparently non-inhibitory levels (Fig. 1), since colonizaton was not further reduced at the higher SP levels (Fig. 2).

There was a logarithmic relationship between dry weight of non-mycorrhizal and VAM plants and extractable P levels from RP and SP (Fig. 3). The slope of the response curve for non-mycorrhizal CC did not differ between P sources. VAM significantly increased the response to added P, but the slope of the line was greater for RP than SP.

Growth of CC was less with SP than RP probably because initial high levels of available P in SP-amended media inhibited VAM colonization and then declined to levels insufficient to support maximum growth. In RP-amended peat-perlite where P-release was controlled, VAM colonization was comparable to that in soil (1), and growth exceeded that of non-mycorrhizal plants fertilized with higher levels of soluble P because P-induced Cu-deficiency was prevented (Fig. 3).

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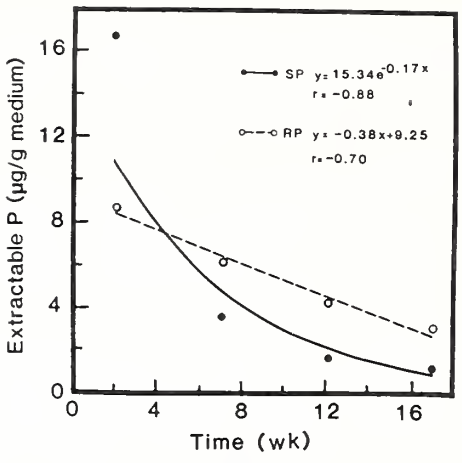

Fig. 1. Mean extractable P levels in peat-perlite after amendment with 4 levels of rock phosphate (RP) or superphosphate (SP).

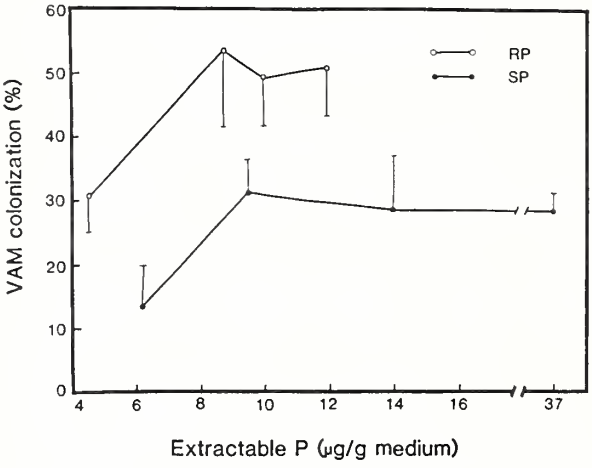

Fig. 2. VAM colonization of 9-mo-old CC seedlings in peat-perlite amended with 4 levels of RP or SP. Extractable P levels measured 2 wk after amendment.

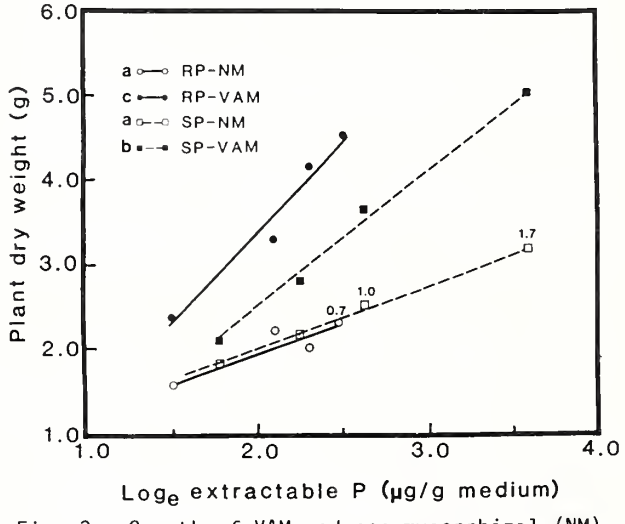

Fig. 3. Growth of VAM and non-mycorrhizal (NM) CC in peat-perlite amended with RP or SP. In legend, lines preceded by different letters have significantly different slopes ($P \leq 0.05$). Treatments with ratings had Cu-deficiency symptoms (1 = mild, 3 = severe).

GROWTH OF MYCORRHIZAL CITRUS OUTDOORS IN CONTAINERS

By

S. Nemec, USDA, ARS, Orlando, FL

Keywords--Glomus, vesicular-arbuscular mycorrhizal fungi

Introduction

Citrus growth in Florida soils was enhanced 1) by inoculation with vesicular-arbuscular mycorrhizal fungi (VAM). In fumigated nursery soils, noninoculated control citrus plants usually become infected by residual VAM populations by the end of the first year of the experiment or during the second year if the test is left in the field that long. Infection from residual inocula makes it difficult to contrast long-term growth of inoculated plants with uninoculated plants.

This experiment was established in drainage tiles containing fumigated soil to study long-term growth of inoculated citrus compared to control plants.

Methods and Materials

Twenty-one clay drainage tiles with interior dimension of 28" X 39" and a perforated cement bottom at the narrowest end were filled with low phosphorus (P = 7 ppm) Astatula fine sand and then fumigated with 1 lb methyl bromide per tile on May 2, 1978. On May 17, seven tiles were each planted with a single rough lemon (Citrus limon L.) seedling preinoculated with Glomus intraradices, seven with rough lemon preinoculated with G. mosseae and seven with a noninoculated control. The experiment was set up in a randomized complete block design with seven blocks. The plants were fertilized with a 16-0-16 granular fertilizer until the test was terminated in 1982 because of freeze damage. Growth data were taken each year. Rough lemon was budded with the Valencia scion in 1979.

Results and Discussion

Growth results show (Table 1 and Fig. 1) that in each year inoculated plants were superior to the controls. This increased vigor may have enhanced plant survival during the January 1982 freeze. In 1982, 3 plants infected with G. intraradices were killed, 2 infected with G. mosseae were killed, but 4 controls were killed. Less severe freezes during 1979 and 1981 caused some leaf injury and twig death and this reduced the overall growth potential during the duration of the test. Plants in 1982, especially those in the G. intraradices treatment, had reached a size large enough to produce a few fruit. By 1982, even the control plant roots had become infected with VAM (Table 2).

This study like many other field and nursery mycorrhizal studies indicates that control plants usually become infected with indigenous VA-fungus species during the course of the tests. Infection in control plant roots could have come from blowing sand and inocula, from sources of inocula in incompletely fumigated sand in the lower end of the tiles, or from outside the bottom of the tiles.

Table 1. Annual growth of rough lemon seedlings inoculated with two Glomus species.

Glomus species	Years				
	1978	1979	1980	1981	1982
	Top growth (cm)				
G. intraradices	66***	96***	99ns	156*	169ns
G. mosseae	62*	95***	101ns	150*	167ns
Control	47	66	83	116	139
	Stem girth (cm)				
G. intraradices	-	6***	6**	11**	13***
G. mosseae	-	6***	6***	11**	13***
Control	-	4	5	9	11

*P = 0.05, **P = 0.01, ***P = 0.001, ns = not significant; treatments compared with control.

Table 2. Glomus vesicles, hyphae, and percentage of fungus infection in rough lemon citrus roots - 1982.

Glomus species	Fungus structures		%
	Vesicles	Hyphae	Infection
G. intraradices	1.09*	1.58ns	89ns
G. mosseae	0.32ns	1.47ns	81ns
Control	0.44	1.48	78

0 = no vesicles or hyphae, 1 = 0-50, 2 = 51-100, and 3 = 100 + vesicles in 1-cm-long root pieces. Hyphae rated 1 to 3, light to extensive, respectively. 100 root pieces sampled from each of 3 plants per treatment.
*P = 0.05, ns = not significant, treatments compared with control.

Figure 1. Drainage tile experiment in November 1981.

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A FLUID-DRILLING APPLICATOR FOR APPLYING VAM IN THE FIELD

By

S. Nemec and J. J. Ferguson

Keywords--citrus, fluid-drilling, field inoculation

Introduction

Use of vesicular-arbuscular mycorrhizal fungi (VAM) in commercial agriculture requires that efficient means be devised to apply inoculum to field plantings. This summary describes an apparatus designed to apply inocula along with seed in a slurry to rows in a field plot, and the results of an experiment in which it was used.

Methods and Materials

The applicator described in this summary is patterned after an original design of a unit built by Mr. George Campbell of the Campbell Soup Co., Cairo, Georgia. His unit was developed to apply vegetable seed in hydrogel slurries under pressure to small plots. Application of seed to soil in this way is called fluid-drilling. Larger mechanized equipment is available for commercial fluid-drilling applications. The unit used in this study was built on a 4'1" X 12" (rear) steel frame. Three balloon tires were mounted on the frame in tricycle fashion. The seed-fungus slurry was dispensed under pressure to a drill from a hose connected to a 5-gal tank. The tank was connected to a pressure regulator on a second tank containing pressurized nitrogen.

This applicator was used to apply Cleopatra mandarin (Citrus reticulata Blanco) citrus seed, soil containing spores, extramatrical hyphae, and root fragments of Glomus intraradices, G. mosseae and G. deserticola, and a hydrogel (Viterra-2) to a nursery seedbed near Dundee, Florida, on May 7, 1982. Inocula of each Glomus species were adjusted so that each treatment replicate received 12,000 chlamydospores. The slurry was made up to contain 0.6% Viterra-2 and 125 seed were applied per treatment replicate. Four replicates per treatment were established and each replicate was 20 feet long. Plant height was taken in the fall of 1982 and 1983, and percentage of root infection determined in 1983. The plot was fumigated with methyl bromide prior to treatment.

Results and Discussion

The quantity of Viterra-2 used was chosen because it provided an aqueous gel with enough viscosity to allow the seed to remain suspended in solution. The applicator was hand-pushed down the row and the drill adjusted so that the slurry was injected about 1" below the soil surface. Leveling irons and packer wheels mounted behind the drill leveled and packed the soil after the slurry was injected into the soil.

Height of the seedlings at the end of 1982 did not differ among treatments (Table 1). In 1983 seedling height in all three fungus treatments was significantly increased over that of the control. Only infection by G. deserticola was significantly greater than the infection in the control. Results of this study and those of Hayman et al. (1) indicate the potential usefulness of fluid-drilling to apply both VAM and seed in a hydrogel.

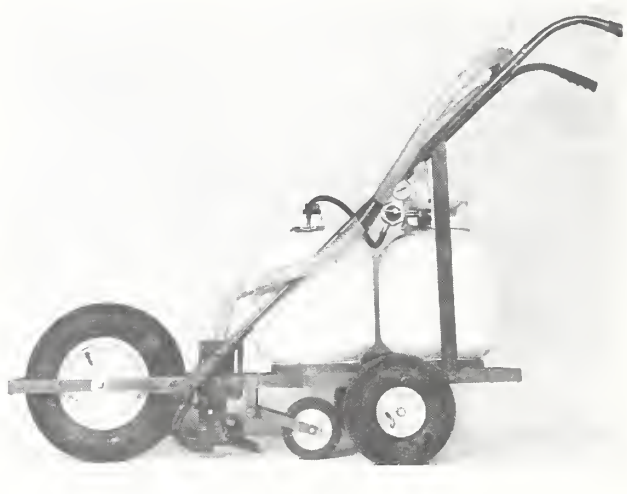

Figure 1. Fluid-drilling applicator

Table 1. Height of Cleopatra mandarin seedlings and fungus infection in roots one and two growing seasons after planting by fluid-drilling in the row a slurry of hydrogel, seed, and three Glomus species.

Treatments	Plant height (cm) 1982	Plant height (cm) 1983	% Fungus infection 1983
Glomus intraradices	23.6ns	41.6***	80ns
Glomus mosseae	13.9ns	23.9***	81ns
Glomus deserticola	21.8ns	39.2***	95*
Control	20.6	30.6	76

Data are means of 4 replicates. Viterra-2 (0.6%) was added to all treatments. Fungus treatment means differ from control: *P = 0.05, ***P = 0.001, and ns = not significant.

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HOW TOPSOIL STORAGE DURING SURFACE MINING INFLUENCES THE INOCULUM POTENTIAL OF MYCORRHIZAL FUNGI

Judith H. Quam

Keywords--Pseudotsuga menziesii, Alnus rubra, nodulation, ectomycorrhizae, VA mycorrhizae

INTRODUCTION

The 12,000 acre Centralia Coal Mine is located halfway between Seattle, WA and Portland, OR. The mine supplies coal for a nearby steam-electric plant. In the course of the surface mining, topsoil is removed and can be stockpiled for as long as ten years while mining is conducted on the site. Washington Irrigation and Development Company contracted this study as part of their reclamation program.

The objective of the study was to determine how stockpile depth and duration influence the inoculum potential of mycorrhizal fungi for Douglas-fir (Pseudotsuga menziesii), red alder (Alnus rubra), and ryegrass (Lolium multiflorum).

METHODS

Samples of stockpiled topsoil from two soil types (Logan Hill, Weathered Skookumchuck) and several depths were taken for three years. The stockpile of Logan Hill topsoil had been dismantled before the third year, so a second two year old stockpile was examined. The soil samples were dried to a manageable consistency and evaluated using a greenhouse bioassay with red alder, Douglas-fir, and ryegrass. Autoclaved soils were used to access greenhouse contamination. Forest soils were assayed as a sample of mycorrhizal potential for a given year. Approximately 750 plants were evaluated each year. Also, soil samples were wet sieved for spores of VA mycorrhizae and sclerotia.

RESULTS

Mycorrhizal propagules declined faster in Weathered Skookumchuck (WS) topsoil than Logan Hill (LH). In both the WS and LH soils the mycorrhizal propagules deteriorated faster with depth (figure 1). Red alder mycorrhizal propagules survived better in stockpiles than did Douglas-fir mycorrhizal propagules (figure 2). Colonization of the WS stockpile by volunteer red alder the third year had a noticeable effect on the surface sample. On Douglas-fir no Cenococcum mycorrhizae were found after the first year. By the third year an important group of dark brown mycorrhizae had been virtually eliminated. Essentially no VA spores or mycorrhizal colonization of ryegrass or red alder were found in either forest soils or stockpiled topsoil.

The nitrogen fixing endophyte of red alder declined substantially the third year. Many nodulated nonmycorrhizal seedlings were found but few unnodulated mycorrhizal seedlings (figure 3).

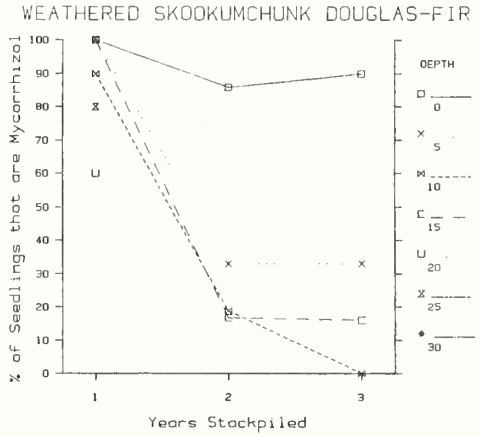

Figure 1. Percent of mycorrhizal Douglas-fir seedlings in stored WS topsoil.

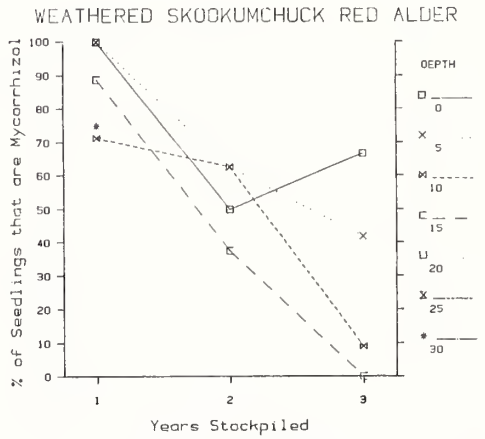

Figure 2. Percent of mycorrhizal red alder seedlings in stored WS topsoil.

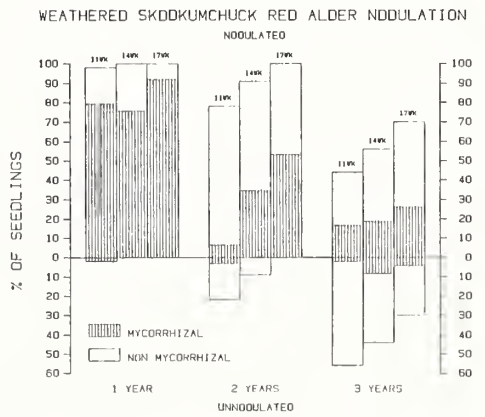

Figure 3. Percent nodulated and mycorrhizal red alder seedlings.

GROWTH OF GLOMUS-INFECTED YELLOW-POPLAR ON COAL SPOIL

By

R. Hay and J.Rennie

Keywords--Glomus, Liriodendron, nursery culture, fertilizer topdressing

Introduction

Yellow-poplar (Liriodendron tulipifera L.) seedlings occasionally colonize strip mine spoil (Rothwell and Vogel, 1982). It is usually not an abundant volunteer; neither is it commonly planted for mine revegetation. Yellow-poplar is a site-demanding species, responding to high quality sites with maximum growth rates which are frequently maintained for much of the life of the tree. Endomycorrhizae might provide yellow-poplar seedlings with sufficient capabilities to survive and grow on sites that are usually considered marginal. Certainly much has been accomplished using selected ectomycobionts with various pines for strip mine revegetation.

It was our purpose to test the suitability of yellow-poplar seedlings that had been grown in nursery culture under various mycorrhizal and fertilizer treatments for reclaimed strip mine revegetation.

Methods and Materials

The outplanting site was at 792 m elevation in the Cumberland Mountains north of Knoxville, TN. It had been mined and reclaimed just prior to experiment establishment; pH was 4.1 to 4.8, phosphorus and potassium levels were low, and nitrogen was very low. The carbonate shale was showing indications of weathering rapidly. Except for some nearby experimental pine plantings, vegetation on the site was lacking when the yellow-poplar was planted. The seedlings had been grown in a nursery for one season using four mycorhizal treatments imposed upon four fertilizer treatments. Glomus mosseae and G. fasciculatus grown on sorghum roots plus humus from a natural, pure yellow-poplar stand were used as mycorrhizal inoculum. Fertilizer treatments included a base application of 15-15-15 followed by variously timed ammonium nitrate topdressings throughout the growing season.

Planting holes (15 cm diameter) were augered into the spoil for a depth of 30 cm to 46 cm. The roots were arranged in the hole and the spoil was replaced without any amelioration. A slight depression was retained around the seedling base to hold precipitation.

Size data from the first four years after outplanting were evaluated by analysis of variance and Duncan's multiple range test.

Results and Discussion

Survival after the first year was not significantly influenced by nursery culture treatments. Although there were some ranking changes between treatments, survival means were not significantly different through the fourth year. Generally survival was quite satisfactory, 80-90 percent after 4 years. There were no significant interactions between the mycorrhizae and fertilizer treatments that influenced survival.

Plot volume index (Marx, et al., 1977) showed that mycorrhizae treatments in the nursery had no significant influence on growth and survival during the first four years. However, PVI for the non-mycorrhizal seedlings was consistently the lowest; G. mosseae-infected seedlings had the greatest PVI average the fourth year.

Nursery fertilizer treatments had a significant impact on PVI until the fourth year; seedlings that did not receive any fertilizer in the nursery had significantly lowest PVI through the second year, but all treatment means were grouped together after the fourth year. Seedlings that received nitrogen topdressings in the nursery consistently had the largest PVI during the first four years in the field. Fertilizer did not significantly interact with mycorrhizae.

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Rothwell, F.M. and W.G. Vogel. 1982. Mycorrhizae of planted and volunteer vegetation on surface-mined sites. USFS, Gen. Tech. Rep. NE-66. 12pp.

Figure 1. Four year old Glomus mosseae infected yellow-poplar that received two nitrogen topdressings in the nursery. The stick is 2 meters tall.

INTERACTION OF GLOMUS INTRARADICES, MELOIDOGYNE INCOGNITA, AND PHOSPHORUS ON COTTON

By

G. S. Smith, R. W. Roncadori, and R. S. Hussey

Keywords-- VA mycorrhizae, microplots, disease interaction, tolerance, resistance

Introduction

Most reports on vesicular arbuscular (VA) mycorrhizae and plant parasitic nematode interactions indicate that host tolerance to the nematode is increased following root colonization by mycorrhizal fungi. Several reports have shown that elevated soil phosphorus levels affect nematode-host relationships similar to VA mycorrhizal fungi. The objectives of this study were: (i) to determine if additional P fertilizer affects nematodes similar to VA mycorrhizae in soil containing sufficient P so as to not severely restrict plant growth or mycorrhizal development and (ii) to determine if similar results are obtained in greenhouse and field microplot experiments.

Methods and Materials

Field microplot studies were conducted in 1982 and 1983 in 48 80-cm dia plots fumigated with methyl bromide. Factorial experiments consisted of two levels of Glomus intraradices (GI), Meloidogyne incognita (MI), and superphosphate (P) in all possible treatment combinations. Treatments were replicated six times in a randomized complete block design. Soil P levels in 1982 were: low P = 40 mg/kg, high P = 80 mg/kg and in 1983: low P = 75 mg/kg, high P = 130 mg/kg. Designated microplots were infested with 400 g soil and root inoculum of GI or 273,000 eggs of MI and planted with the root knot susceptible cotton cultivar Stoneville 213. Plant height, nematode reproduction, and mycorrhizal root colonization were measured at monthly intervals and seed cotton yields were recorded at harvest. Similarly designed greenhouse studies were conducted in 1983.

Results and Discussion

In 1983, seed cotton yields in microplots were not affected by P rate except that yields of MI-infected plants in high P soil were lower than MI-infected plants in low P soil (Table 1). Similar trends were observed in 1982. Yields were increased 33% in GI-inoculated plants and reduced 31% in GI+MI plants and 63% in MI plants compared with noninoculated plants. Plant height at 60 days was greatest in GI plants and least in MI plants (Fig. 1). Significant reductions in MI juveniles/100 cm^3 soil due to colonization by GI were observed at 60 days after planting when data were averaged over P rate. However, at harvest no significant reductions in MI reproduction was observed due to GI, but root colonization by GI was suppressed by MI.

In the greenhouse, fresh boll weights were similar for GI- and GI+MI-inoculated plants and were significantly greater than controls or MI-infected plants at either P rate (Table 1). GI suppressed MI egg production 62% and juveniles 85% when averaged over the P rates.

Greenhouse data did not corroborate results obtained from field microplots. Although similar MI inoculum levels were applied at both locations, nematode damage was much greater under field conditions. We conclude that in the greenhouse, GI rendered cotton less suitable for MI and added P enhanced plant tolerance. In field microplots where plant growth was more severely affected by MI, GI apparently increased plant tolerance whereas added P enhanced plant susceptibility to the nematode.

TABLE 1. Influence of Glomus intraradices, Meloidogyne incognita, and superphosphate on yields and nematode reproduction in 1983 field microplots and greenhouse tests.

	Microplots		Greenhouse	
Trt	Seed cotton g/plot	60 day juveniles	Boll wt. (g)	Eggs/ g root
CK[v]	161[w]	–	74[y] d	–
CK+P	171	–	77 cd	–
GI	195	–	86 a	–
GI+P	208	–	nd	nd
MI	89	241[x]	65 e	1016[y] a
MI+P	49	346	74 d	950 a
GI+MI	121	132	84 ab	456 b
GI+MI+P	121	189	80 bc	293 b

[v] CK=control, GI=Glomus intraradices, MI= Meloidogyne incognita, P=superphosphate

[w] $LSD_{0.05}$=30 for treatment means averaged over P rate.

[x] $LSD_{0.05}$=93 for treatment means averaged over P rates.

[y] Numbers in each column followed by the same letter are not statistically different at P=0.05.

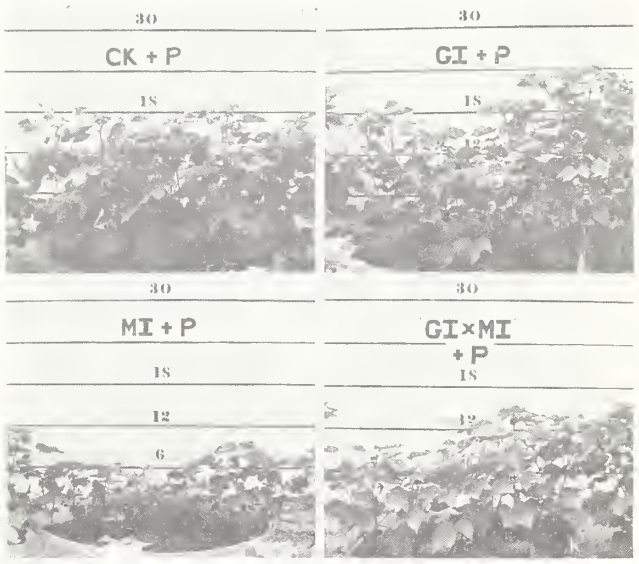

Figure 1. Plant heights of 60 day old cotton in treatments at high phosphorus 9130 mg/kg soil) from 1983 microplots.

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INTERACTIONS OF GLOMUS INTRARADICES, MELOIDOGYNE INCOGNITA, AND FUSARIUM OXYSPORUM f.sp. VASINFECTUM RACE 1 ON COTTON

By

G. S. Smith, R. W. Roncadori, and R. S. Hussey

Keywords-- VA mycorrhizae, Fusarium wilt, root knot nematode, synergism

Introduction

Since Atkinson's report in 1892 on the association between Fusarium wilt severity in cotton and root knot nematodes, there have been few reports in the literature quantifying this synergistic interaction. Yang et al. reported Meloidogyne incognita and Belonolaimus longicaudatus to be the primary nematodes contributing to wilt severity. Recently, several researchers have reported that vesicular-arbuscular (VA) mycorrhizae interact with Fusarium on tomato, and Verticillium on cotton. Previous work by the authors has shown that VA mycorrhizae reduce nematode damage on cotton. The objective of this study was to test the effects of the endomycorrhizal fungus Glomus intraradices on the Fusarium wilt-root knot nematode complex in cotton.

Methods and Materials

The root knot nematode susceptible cultivar Stoneville 213 was grown in fumigated soil (pH 5.8, phosphorus 62 mg/kg soil) free of or infested with G. intraradices (GI) (1000 spores/pot), five conidial inoculum densities of Fusarium oxysporum f.sp. vasinfectum race 1 (FOV) (0,5,50,500,5000 c.f.u./g soil), and four inoculum densities of Meloidogyne incognita (MI) (0,188,375,750 eggs/100 cm^3 soil) in all possible combinations in a greenhouse factorial experiment. Fresh boll weights, nematode reproduction, Fusarium root infection, and mycorrhizal root colonization were measured 90 days after emergence. Analysis of variance and regression analysis were performed on the data.

Results and Discussion

GI increased fresh boll weights by 30% compared with noninoculated plants when averaged over all levels of nematode and fusarium (Table 1). MI decreased boll weights 13%, 22%, and 30% when averaged over low, medium, and high inoculum levels, respectively. FOV decreased boll weights 5%, 17%, and 57% at 5, 50, and 500 cfu/g, respectively. MI x FOV interaction results suggest that boll weights are unaffected until initial soil infestation levels of FOV reach 50 cfu/g with medium MI inoculum levels. Boll weight reductions of approximately 20% occurred with FOV levels of 50, 500, and 5000 cfu/g at medium, zero, and low MI inoculum levels respectively. Boll weights were reduced 86% at 500 cfu/g FOV and high MI levels.

Forty-three percent of roots were infected by FOV in plants inoculated with GI compared with 49% for noninoculated plants when averaged over all inoculum levels of nematode and fusarium. Percentage of roots infected by FOV were 6%, 21%, 57%, 92%, and 90% at initial soil levels of 0, 5, 50, 500, and 5000 cfu/g, respectively. Percent mycorrhizal root colonization was reduced at 500 cfu of FOV only when MI was also present.

MI eggs/g root averaged 581 for noninoculated plants compared with 228 for GI inoculated plants. Nematode reproduction increased from 406 eggs/g root at the low MI level to 652 and 667 at medium and high MI levels, respectively. Similarly, increasing FOV inoculum densities of 0, 5, 50, and 500 cfu/g resulted in 299, 389, 439, and 626 eggs/g root, respectively. Significant interactions occurred between MI x FOV, GI x FOV, and GI X MI. In general, nematode reproduction was stimulated by increasing inoculum levels of MI and FOV with GI reducing egg production from 42% to 86% depending on the inoculum levels of MI or FOV.

Table 1. Analysis of variance for fresh boll wt., percent roots infected by Fusarium, mycorrhizal root colonization and eggs/gram root.

Source	df	Boll weight	% roots infected with FOV	Mycorrhizal root colonization	Eggs/g root
Gi[x]	1	****	*	****	****
MI[y]	3	***	ns	ns	****
FOV[z]	4	****	****	****	**
Gi*Mi	3	ns	ns	ns	**
Gi*FOV	4	ns	ns	ns	**
MI*FOV	9	**	ns	**	****
Gi*Mi*FOV	9	ns	ns	ns	ns

*=.05, **=0.01, ***=0.001, ****=.0001
x=Glomus intraradices, y=Meloidogyne incognita, z=Fusarium oxysporum f.sp. vasinfectum race 1

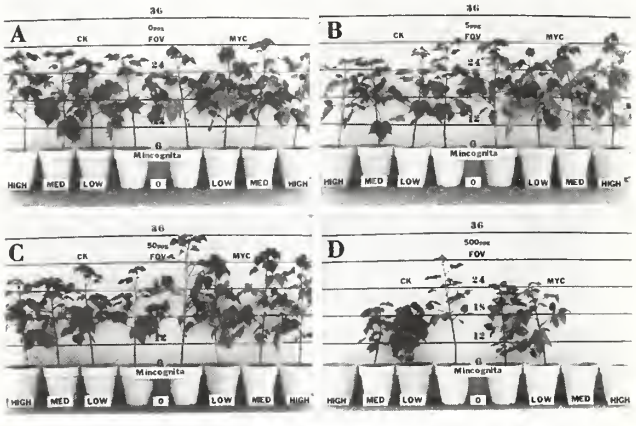

Figure 1. Stoneville 213 cotton plants without (CK) or with G. intraradices (MYC), four inoculum levels of M. incognita (0, Low, Med, High) and four inoculum levels of Fusarium oxysporum f.sp. vasinfectum (FOV). A. 0 cfu/g, B. 5 cfu/g, C. 50 cfu/g, D. 500 cfu/g.

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VA MYCORRHIZAE AND RECLAMATION OF ARID AND SEMI-ARID LANDS

By

S. E. Williams and M. F. Allen

Preface

During the Fifth North American Conference on Mycorrhizae held in Quebec City, Canada (August, 1981), several people from the Intermountain and Rocky Mountain Region of North America met informally to discuss problems in arid land reclamation particularly as related to vesicular-arbuscular (VA) mycorrhizae. The group met several times under numerous conditions, and it was determined during the course of these gatherings that a workshop should be held the summer of the following year to bring together scientists from the region in question in an informal setting for discussion of VA mycorrhizae and reclamation of arid and semiarid lands. D. L. Lindsey, of New Mexico State University, initiated the concept of having the meeting and somehow appointed S. E. Williams and M. F. Allen to organize the meeting.

The meeting commenced late on August 17, 1982, with an informal assemblage and discussion. August the 18th was devoted to oral presentation of papers and discussion while the 19th was reserved for a taxonomy and staining workshop. This document contains papers presented on August 18, 1982, as well as poster abstracts.

VA mycorrhizae and land reclamation continues to be an area of much research. However, directing information on this topic into the hands of mine managers and reclamation personnel is a perplexing problem. It is hoped that the volume which resulted from this meeting may provide a mechanism whereby such information can reach reclamation clientele. Our expectation is also that this writing will be useful to researchers in many areas of V.A. mycorrhizae work.

This fairly small document is the work of a rather large constituency of Mycorrhizasts. Certainly the authors and conference participants have contributed much; however, others, particularly reviewers and typists have had much to do with the quality of this volume.

Summary

There are many theoretical problems to be overcome before the potential of VAM use in reclamation is realized. First and foremost is perception. All microorganisms, including VAM fungi, live in a world almost imperceptable to us. The universe to a microbe may be, at best, limited to a few centimeters. Thus, disruption of a soil unit the size of a badger mound may, to a fungus, be as intensive as a stripmine is to a man. Obviously soil microbes must be adept at dealing with disturbances. The second problem encountered is mechanism of physiological interaction. One must have a background in soils, plant physiology and fungal physiology to understand how VAM work. However, it is inconceivable that one mechanism of interaction, hyphal transport of P can account for all physiological responses noted in all soil conditions from all biomes. Physiological studies and interpretation beyond the correlative stage are needed. Thirdly, there is a major lack of field studies on establishment in any habitat. Only one field study - has been reported in the Rocky Mountain region and few in other areas. The need for field studies is essential for those involved in reclaiming disturbed areas. Fourth, the possibility that mycorrhizae may act as a vector for succession is merely correlative; experimental study remains essential.

Acknowledgments

Special thanks are due to several people: G. E. Schuman and M. Christensen for their reviews of the entire manuscript, D. M. H. Watson for her critical assistance in coordination of the Dubois conference, and Sharon Johnson who typed the manuscript.

Contents

Copies of the Symposium may be obtained by writing S. E. Williams, at Plant Science Division, University of Wyoming, Laramie, Wyoming 82071.

EFFECT OF GLOMUS ETUNICATUM AND NATIVE VAM FUNGI ON DIRECT-SEEDED AND TRANSPLANTED GREEN ONIONS

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Keywords -- Allium cepa x Allium fistulosum, soil fumigation, low P

Introduction

An experiment was designed to determine the response of direct and transplanted green onion, Allium cepa x Allium fistulosum 'Beltsville Bunching', to Glomus etunicatum (GE) inoculation in low phosphorus soil in South Carolina with the following objectives: 1) to compare the infection levels of GE, native VAM fungi, and GE + native VAM fungi, 2) to determine uptake of P and other nutrients and 3) to determine overall growth responses to an introduced single strain of GE as opposed to the native endophytes.

Methods and Materials

Soil samples obtained from non-fumigated plots were evaluated for native populations and densities. The soil was fumigated with methyl bromide at 976 kg CH_3Br (methyl bromide 98%, chloropicrin 2%) per hectare one month before seeding or transplanting. Spores of GE was increased in greenhouse pot culture with corn serving as the nurse crop. The inoculum was applied by mixing the appropriate quantities of GE spores to soil scooped out of the planting furrow (7.5 x 10 x 120 cm) and returning the soil + inoculum to the furrow. Soil from appropriate plots was collected to raise transplants in the greenhouse.

The soil pH at planting was 5.6 and N, Ca, K, S and B were applied to meet the recommended levels by the South Carolina Extension Service. The soil P at planting was 18 kg/ha.

Results and Discussion

There was a low amount of native mycorrhizal fungi (0.5 spores/g) and the composition was dominated by GE (52%) and other Glomus spp. (24%). Despite the paucity of the native mycorrhizal spores noted, the infection rating was generally high for the non-fumigated control (Table 1).

Significant differences between treatments were noted in all growth parameters (Table 1). Also, the increases in growth parameters corresponded to mycorrhizal infection and were highly correlated (P = 0.0001).

Results (Table 2) show that inoculation with GE had a profound effect on uptake of all the nutrients. GE inoculation plus native VAM fungi treatment was significantly better for uptake of nutrients than the native endophytes alone. Similar responses were observed in fresh weight per plant, Zn, and Cu.

Table 1. The effects of GE inoculation and CH_3Br treatments on the root colonization by VAM hyphae and growth components of the direct-seeded onions 19 weeks after seeding.

CH_3Br Fume	GE Inocul.	Dry Wt. g/plant	Bulb Diam. cm/plant	Yield m tons/ ha	Myco. infect. (0-4)
+	+	9.6	2.11	56.06	3.7
-	+	8.9	2.25	45.21	3.9
+	-	1.9	0.83	9.55	0.5
-	-	5.2	1.66	39.83	3.4
LSD .05		0.9	0.12	5.49	0.2
Myco. corr.		***	***	***	
Myco. corr.		0.74	0.80	0.84	

See footnotes with Table 2.

Table 2. The effects of GE inoculation and CH_3Br fumigation treatments on the uptake of nutrients in the direct-seeded onions nineteen weeks after seeding.

CH_3Br Fume	GE Inocul.	Total Nutrient Uptake			
		N	P	K	S
		(mg/plant)			
+	+	356.88	16.37	176.54	19.54
-	+	330.77	24.40	24.40	23.38
+	-	73.97	3.02	3.02	3.41
-	-	213.57	10.86	10.86	11.17
LSD .05		90.07	9.83	69.59	6.17
Myco. corr.		**	**	**	**
Myco. corr.		0.81	0.67	0.63	0.76

GE + = 1 spore/g at seeding
- = 0.0 spore/g at seeding
CH_3Br fumigation: + = plus fumigation
- = no fumigation
* ... Significant at P = .05
** ... P = .01
*** ... P = .0001

Similar responses to GE inoculation and CH_3Br fumigation were observed for transplanted onions. All the mycorrhizal plants were significantly better than non-mycorrhizal plants (P = .05). Treatment effects generally were lower than for the direct-seeded plants in comparable treatments.

The high infection rate of 3.4 by the native VAM fungi found in the direct-seeded onions showed that the native mycorrhizae were virulent but not as effective as the introduced strain GE (Tables 1 and 2). These results also demonstrated that addition of an effective VAM fungi to a soil containing low or ineffective VAM fungi greatly improved the uptake of nutrients and growth of onions.

SPORE PRODUCTION BY FIVE VAM FUNGI AS INFLUENCED BY PLANT SPECIES

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Keywords - Glomus claroideum, Glomus etunicatum, Glomus mosseae, Glomus macrocarpum, Gigaspora margarita, Zea maize Glycine max, Paspalum notatum, Sorghum vulgare, nurse crops.

Introduction

Endomycorrhizal fungi spore production is accomplished by the use of a nurse crop. This is a vital aspect of all studies dealing with VAM Fungi. Studies by various researchers have been conducted to evaluate factors (soil temperature, light intensities, and plant stress) that govern spore production. However, these studies have usually been limited to the use of one mycorrhizal fungal species or one plant species. The objective of this experiment was to examine four plant species for use as nurse crops for spore production by five mycorrhizal fungi.

Methods and Materials

The soil used for this experiment was a Vaucluse loamy sand (pH = 6.0, phosphorus level = 3 µg/g). The soil was autoclaved three times for 90 minutes each time. Mycorrhizal fungal spores of either Glomus claroideum, G. etunicatum, G. mosseae, G. macrocarpum or Gigaspora margarita were added to 1,000 g of soil at a rate of 1 spore/g. The soil and spores were placed in a plastic bag, mixed, then placed in a 11 x 14.5 cm plastic pot. The plant species used were corn (Pioneer 3369A, 2 plants/pot), soybean (Bragg, 2 plants/pot), bahiagrass (25 plants/pot) and sudangrass (25 plants/pot). The pots were placed in a growth chamber set at 27-18C, day--night with light intensity of 275 $\mu E/m^2/sec$. The plants were watered daily to 100% field capacity. Whole pots were removed at 6, 8, 10, 12 and 14 weeks after planting. At harvest the root/soil mixture was chopped up, placed in plastic bags then stored at 4C. Two 10g soil samples were wet-sieved to obtain mycorrhizal spores for counting.

Results and Discussion

Spore production by mycorrhizal fungi was influenced by the plant species used as the nurse crop. As was anticipated, spore production increased from week 6 to 14 for all combinations with G. mosseae x soybeans, G. claroideum x soybeans, G. etunicatum x soybeans and G. macrocarpum x corn showing no significant (5% level) differences over time. Mycorrhizal spore production for most crop species by mycorrhizal fungi peaks at weeks 12 and 14. Tables 1 and 2 show the mycorrhizal spore numbers per gram of soil for weeks 12 and 14, respectively. At week 12 for G. claroideum and G. margarita corn was significantly better than the other plant species. At week 14 bahiagrass was significantly better than the other plant species except for G. claroideum, where corn and bahiagrass were not different.

For maximum spore production: Corn for 12 weeks was the best for G. claroideum. Bahaiagrass for 14 weeks was the best for G. etunicatum, G. mosseae, G. macrocarpum, and G. margarita. Soybeans did not promote adequate spore production when used as a nurse crop.

Table 1. Mycorrhizal fungi spore production at week 12 as influenced by plant species.

Species	Crop Species: Corn	Soy-bean	Sudan-grass	Bahia-grass	LSD .05
	(no. spores/g soil)				
Gc	85.8	4.3	12.6	13.1	13.0
Ge	133.4	41.1	57.5	113.8	69.1
Gm	2.1	0.7	0.6	7.3	5.6
G mac	27.9	10.6	2.8	39.8	20.2
G marg	8.4	4.8	6.5	6.0	1.6

Table 2. Mycorrhizal fungi spore production at week 14 as influenced by plant species.

Species	Crop Species: Corn	Soy-bean	Sudan-grass	Bahia grass	LSD .05
	(no. spores/g soil)				
Gc	88.5	1.2	26.8	94.4	38.7
Ge	124.4	22.1	80.1	278.4	24.5
Gm	2.4	1.1	1.2	20.7	6.4
G mac	24.5	7.8	3.8	47.6	12.1
G marg	5.7	4.9	10.0	12.5	3.9

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RESPONSE OF FOUR SOYBEAN CULTIVARS IN FUMIGATED MICROPLOTS TO INOCULATION WITH GLOMUS CLAROIDEUM (VAM FUNGUS).

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Keywords--Glycine max, methyl bromide, specificity

Introduction

Specificity between crop plants and VAM fungi has received considerable attention and to date, specificity or preferential selection by the plant or fungus has not been identified for the systems investigated. Since comparable studies with soybeans [Glycine max (L.) Merr.] and VAM fungi were absent in the literature, we initiated an experiment to evaluate the host : fungal specificity between four soybean cultivars and Glomus claroideum after CH_3Br fumigation of Cecil soil in South Carolina.

Materials and Methods

Microplots consisted of 91.5 cm diameter bins that contained Cecil soil. Plots were fumigated with CH_3Br at 1725 kg/ha and then aerated manually twice over 3 to 4 weeks. The soil was removed from a zone of 10 cm wide by 15 cm deep by 61 cm long: ∿ 10,000 g of soil. Inoculum (+) consisted of 40,000 spores of G. claroideum with chopped corn roots and soil (400 g). Controls (-) for each cultivar received 400 g of soil and chopped non-mycorrhizal corn roots--no spores. After mixing thoroughly, soil was returned to the bins, planted with Bragg, Davis, Lee-74, or Ransom soybean cultivars, and inoculated with Rhizobium japonicum USDA strain 110 granular. Plants were harvested at mid-bloom to early pod-fill (R-2 to R-3) for measurements of top dry weight, root fresh weight, nodule fresh weight, height,stem diameter, and root infection.

Results and Discussion

Top dry weights of the soybean cultivars were significantly (P = 0.05) and consistently (12/12 comparisons) increased by inoculation with G. claroideum in fumigated microplots as anticipated. The increase from inoculation ranged from 28 to 202%. The average % increase by cultivar was: Bragg = 90; Davis = 103; Lee-74 = 118; and Ransom = 125. The absolute increase (g/2 plants) ranged from 43.5 to 52.3 for these cultivars (Table 1). Increases were also recorded for root fresh weight, nodule fresh weight, height, and stem diameter, but these were less consistent than top dry weight responses. Mycorrhizal infection ranged from 44 to 55% in 1980; 33 to 56% in 1981; and 88 to 100% in 1982 for inoculated treatments. Controls had infection of 0 to 22% in 1980; 0 to 33% in 1981; and no infection was observed in 1982. A strong relationship was indicated between root fresh weight of the non-inoculated plants and the subsequent % increase in top dry weights with inoculation. Ransom had the smallest average root fresh weight over 3 years in the fumigated microplots. In contrast, Bragg had the largest average root fresh weight. These results would suggest that soybean cultivars with small root systems (inherent, disease, nematode, chemicals) would be more responsive and benefit more from VAM fungi than cultivars with larger root systems. The increased "root surface" via the hyphae from the VAM fungi would be a major contribution to plants with small root systems.

Our results indicated some specificity amongst soybean cultivars and Glomus claroideum. This "specificity" may be related more to the inherent size of soybean root systems than to some means of selection by the crop or fungus. Based on enhanced growth from inoculation with G. claroideum, cultivars would be ranked: Ransom > Lee-74 > Davis > Bragg.

Table 1. Yearly and average top dry weight responses of soybean cultivars to inoculation with Glomus clariodeum.

Cultivar	Response	1980	1981	1982	Avg.
Bragg	% Inc.	28	65	178	90
	g/2pl.	6.5	25.9	124.6	52.3
Davis	% Inc.	64	60	184	103
	g/2pl.	10.2	20.6	115.7	48.8
Lee-74	%Inc.	69	172	114	118
	g/2pl.	15.4	44.4	70.6	43.5
Ransom	% Inc.	75	99	202	125
	g/2pl.	13.1	30.7	103.7	48.9

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INFLUENCE OF GLOMUS CLAROIDEUM (VAM FUNGUS) AND PHOSPHORUS LEVELS ON SOYBEAN GROWTH IN FUMIGATED MICROPLOTS.

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Keywords--Glycine max, methyl bromide

Introduction

Endomycorrhizal fungi are generally reported to be more beneficial to crop plants in soils with a low phosphorus status than in soils with abundant phosphorus. Since most agronomic and horticultural crops are grown in soils with high levels of phosphorus, a 2-year study was initiated to examine the growth response of soybeans [Glycine max (L.) Merr.] to inoculation with Glomus claroideum after CH_3Br fumigation of Cecil soil with two levels of phosphorus in South Carolina.

Materials and Methods

Microplots consisted of 91.5 cm diameter bins that contained Cecil soil at 25 or 50 kg-P/ha. Plots were fumigated with CH_3Br at 1725 kg/ha and then aerated manually twice over 3 to 4 weeks. The soil was removed from a zone of 10 cm wide by 15 cm deep by 61 cm long: ∿ 10,000 g of soil. Inoculum (+) consisted of 40,000 spores of G. claroideum (4 spores/g soil) with chopped corn roots and soil (400 g). Treatments were also included with spores alone; spores plus chopped non-mycorrhizal corn roots in 400 g soil; and spores plus 400 g of sterile nursery soil (Dothan). Controls (-) received 400 g of soil and chopped non-mycorrhizal corn roots--no spores. After mixing thoroughly, soil was returned to the bins, planted with Bragg soybean cultivar, and inoculated with Rhizobium japonicum USDA strain 110 granular. Plants were harvested at mid-bloom to early pod-fill (R-2 to R-3) to measure top dry weights and root infection.

Results and Discussion

Since all four methods of inoculation gave similar increases in top dry weight, they have been averaged for presentation (Table 1) and discussion. Infection in 1981 ranged from 78 to 100% at both phosphorus levels. In 1982, infection ranged from 16 to 67% at 25 kg-P/ha and 100% at 50 kg-P/ha. Controls were free of infection.

Under limited rainfall in 1981, G. claroideum increased the top dry weight of Bragg soybeans by 76% at 25 kg-P/ha and only 7.8% at 50 kg-P/ha. Whereas in 1982 under more adequate rainfall, the response to inoculation with the VAM fungus was 210% at 25 kg-P/ha and 180% at 50 kg-P/ha.

The presence of G. claroideum with 25 kg-P/ha was equivalent to the 50 kg-P/ha (no mycorrhiza) in 1981 under "drought stress" (32 vs 32 g/plant-Table 1). Under more optimum conditions in 1982, the soybean top dry weight from G.claroideum with 25 kg-P/ha was more than twice the weight obtained with 50 kg-P/ha without the aid of VAM fungi (118 vs 51 g/plant). Our results indicated that the influence of G. claroideum on soybean growth under two levels of phosphorus was moderated by other environmental factors--rainfall. A 3-way interaction (mycorrhiza by phosphorus by environment) appears to govern the outcome of these studies. Thus, a systems approach would help advance studies on the roles of mycorrhizal fungi in crop production.

Glomus claroideum was beneficial to soybean growth even in the presence of a relatively high level of soil phosphorus.

Table 1. Top dry weight of Bragg soybeans after inoculation with Glomus claroideum in Cecil soil with 25 or 50 kg-P/ha.

	Top dry weight			
	25 kg-P/ha		50 kg-P/ha	
Treatment	1981	1982	1981	1982
	g/plant			
Control(-)	18	38	32	51
Mycorrhiza (+)	32	118	34	143
% Increase with Mycorrhiza	76	210	7.8	180

1981 - limited rainfall.
1982 - more optimum rainfall.

The perforated soil system, a new method of root research.

By

T. Limonard and W. Smits [+]

Keywords--root research method.

The perforated soil system is a non-destructive root research method. The system is based upon the fact that roots can grow through perforations in the soil, like worm holes or mole tracks, without significant disturbances.

Specially constructed root boxes are filled with soil (Fig. 1). Through this soil clod parallel horizontally perforations are made at regular distances. Roots do not show abnormal branching or other abnormalities, if the diameter of the perforations is less than 15 mm. The boxes are constructed in such a way that in the perforations air-humidity is kept constantly high and that light is excluded.

With the aid of an intrascope (a kind of endoscope) development of roots and formation of mycorrhiza can be followed either directly or on a TV monitor. Samples can be taken from single roots or mycorrhiza for further observation, by means of biopsy forceps (Fig.2).

Data obtained from regular observations of the holes can be fed in a computer for constructing models.

Undisturbed profiles can be taken from the field with special apparatus and transferred into root boxes for perforation.

Up till now the method has succesfully been used in studies concerning root competition, root architecture, mycorrhizal development, inoculation of single roots with mycorrhizal fungi and post-planting effects of containers on nursery stock.

The root boxes and subsidiary equipment are produced and sold by Mechalectron International B.V., P.O.B. 2088, 2800 BE Gouda, The Netherlands. Tel.: 01820-33255.

Literature :

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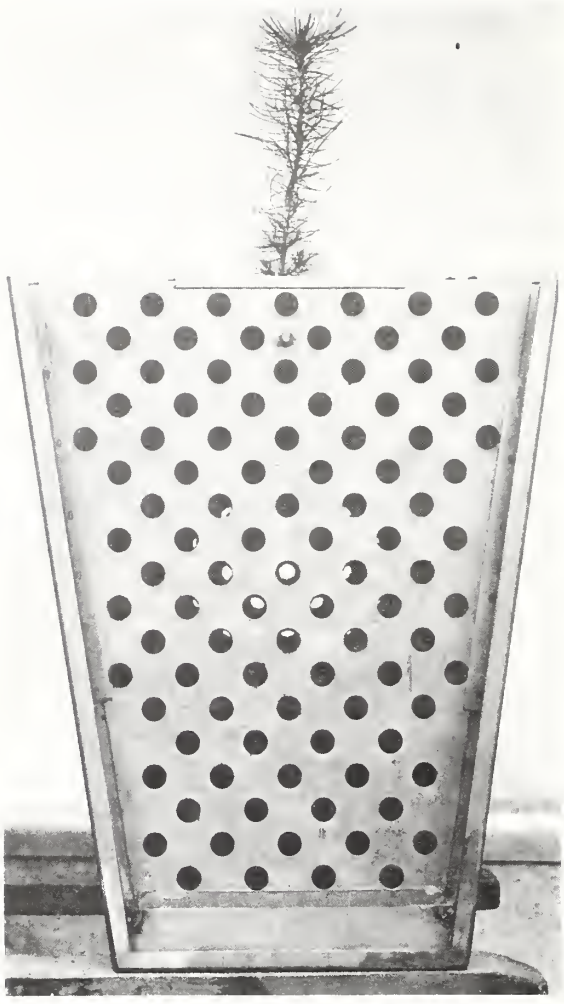

Figure 1. : The "Schalk root box".

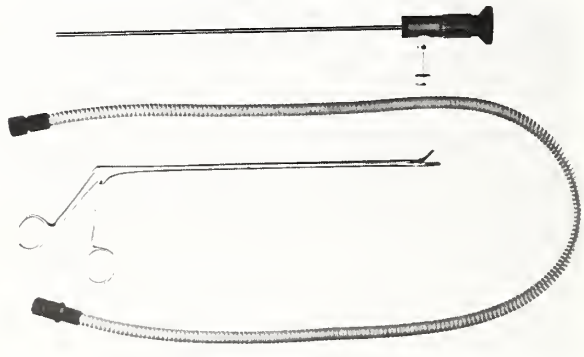

Figure 2. : Intrascope, light conductor and biopsy forceps used for the perforated soil system.

IMPORTANCE OF INOCULUM POTENTIAL OF VAM FUNGI

By

T. Limonard, and W. Smits

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Keywords-- Glomus fasciculatum, G. occultum, Trifolium pratense

Introduction

Inoculum potential (IP) is an important factor determining initial VAM development. It has been little studied due to difficulties of measurement. In this experiment the effect of two VAM fungal species on red clover was examined at five different inoculum potentials at three levels of available soil-phosphorus.

Materials and methods

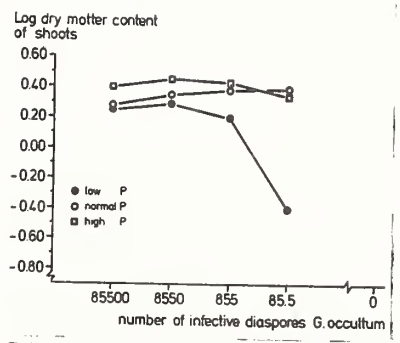
VAM-fungi: Glomus fasciculatum and G. occultum.
Host plant: Trifolium pratense cv. Barfiola.
Soil: sandy loam. pH 7.2, at three levels of water soluble Phosphorus (Pw 3, low; Pw 30, normal and Pw 45, high). Pots used contained 750 ml of soil.
Inoculum: finely cut up and sieved pot balls of mycorrhizal tomato plants diluted with sterile soil. The five inoculum potentials used differed from each other by a factor 10. The original IP of both fungi was determined by a most probable number (MPN) method.
Sampling: at 5, 8 and 11 weeks after sowing each time five pots per treatment.

Results

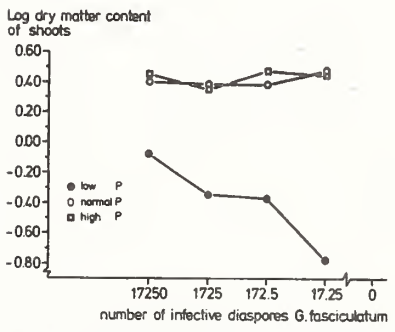
The IP of the original inoculum of G. occultum turned out to be five times that of G. fasciculatum. Data were interpolated to allow for comparisons at inoculum potentials with the same number of infective diaspores. The development of infected root length (VAM-length) increased with time. The slope was steeper for each higher IP. For G. occultum it was much steeper than for G. fasciculatum. The VAM length after 11 weeks at the lower comparable IP (85 infective diaspores per pot) was much higher for G. occultum than for G. fasciculatum. It also increased with soil P. At around 17250 infective diaspores per pot there was still a large although smaller difference between the VAM length of both species, but hardly any difference at normal and high P levels. The VAM length of G. occultum did not differ at this IP for the three P levels. At still higher IP the VAM length with G. occultum tended to decrease at all P levels. This was not so for G. fasciculatum. The dry matter content of the clover shoots increased with IP for G. fasciculatum at low P for G. occultum the increase leveled off at higher IP.
At normal and high soil P there was practically no effect on dry weight of shoots after 11 weeks. The observed tendency to decrease slightly at higher IP was not found to be statistically significant.

CONCLUSIONS

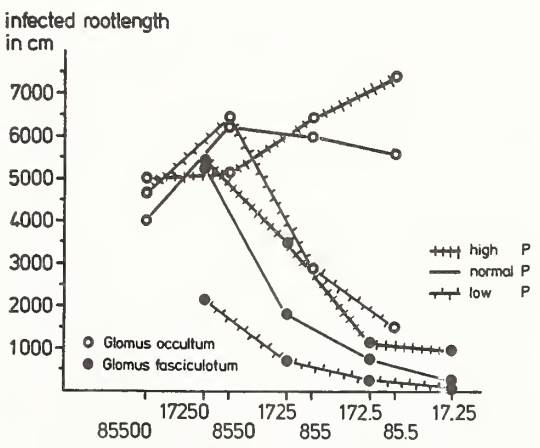
IP of VAM fungi is especially very important during the first weeks of growth (crops with short growing season) and in comparing the effectiveness of species and isolates. There is a definite interaction with soil P level.



Relation between I P of G. occultum and dry matter content of clover shoots after 11 weeks.



Relation between I P of G. fasciculatum and dry matter content of clover shoots after 11 weeks.



Relation between I P of both fungi and infected root length after 11 weeks.

NUTRIENT UPTAKE BY COFFEE INOCULATED WITH GIGASPORA MARGARITA

By

E.S. Lopes, R. Dias, S.V. Toledo, R. Hiroce and E. Oliveira

Keywords - Coffea arabica, phosphorus, zinc mycorrhizal dependency

Introduction

Coffee roots are normally colonized by VAM mycorrhizal fungi and over 40 spore types were observed in the rhizosphere of this plant in the State of São Paulo, Brazil. (Lopes et alii, 1983a).

The fungus G. margarita is efficient in promoting growth of coffee seedlings. (Lopes et alii, 1983b). Phosphorus is deficient in most coffee areas and zinc is deficient in areas with sandy soils. Two experiments were carried out to verify the degree of dependency of coffee seedlings to G. margarita in the uptake of phosphorus and zinc.

Methods and Materials

In one experiment the cultivar "mundo novo" CP 379-19 was cultived in pots with 1.5 kg of a Red Yellow Latosol fertilized with increasing phosphorus (0; 16.5; 32.5; 65.0 and 130.0 mg P/kg of soil) and inoculated or not with the fungus Gigaspora margarita. In the other experiment zinc was added in increasing dosis (0; 0.625; 1.25; 2.50 and 5.0 mg/kg of soil). The soil used had pH of 5.1 and 4.0 µg P/cm^3 of soil and was gamma irradiated (2.5 Mrads), limed (2.0g of finelly grounded dolomite) and fertilized. Phosphorus was supplied as triple superphosphate mixed with the soil; zinc was added as solution of $ZnSO_4$. The inocula was obtained from a mother culture of Macroptilium atropurpureus and was composed of bits of colonized root plus spores in the soil. It was added to the germinating bed and also to the seedling transplanting hole. Harvest was at 240 days after transplanting. Total phosphorus was determined by the vanadate-molybdate yellow method and zinc by atomic absorption spectrophotometry. Length of root colonization was done by the grid plate method (Ambler and Young, 1977), after staining. Spore counting was done after wet sieving (Gerdemann and Nicolson, 1963).

Results and Discussion

There has been no responses to additions of phosphorus in the not inoculated treatments, but clear responses were observed in all levels of phosphorus in the inoculated ones. The inoculation by itself promoted an increase in the dry matter production of 3.5 times the value of not inoculated control treatment and this was associated with increase in P uptake (figure 1). There has been no response to addition zinc (table 1). In both experiments the leaf contents of P, Zn and Cu were higher in the inoculated plants but K, Ca, Mg and Mn were lower. The

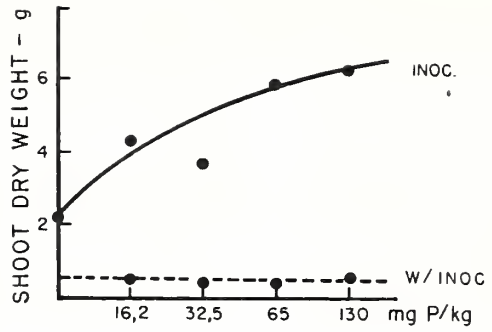


Figure 1. Growth response of coffee seedlings not inoculated (w/inoc) or inoculated (inoc) with G. margarita to added phosphorus.

amount of spores of the fungus were higher in the soil with added P, but not in the one with added zinc. Coffee has shown to be a plant highly dependent on mycorrhizae for P uptake.

Table 1. Growth response of seedlings not inoculated or inoculated with G. margarita to added Zn.

Zinc added to soil mg/kg	Shoot dry weight	
	without inoculation	G. margarita
	g	g
	0.59	6.03
0.625	0.53	5.92
1.250	0.58	6.30
2.500	0.50	5.80
5.000	0.55	5.92

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USE OF COMPONENTS OF VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI AS INOCULUM

By

Pathmaranee Nadarajah,* A.Nawawi, and B.C. Stone

Keywords - Glomus fasciculatum, Acaulospora scrobiculata, Gigaspora margarita, Elaesis guineensis, Theobroma cacao, infection.

Introduction

Different species of vesicular-arbuscular (VA) mycorrhizal fungi and strains of a species differ in their infectivity and capability. Various inoculum preparations such as soil inoculum (spores, soil, hyphae and infected mycorrhizal roots) spore inoculum and mycorrhizal root inoculum, have been used to stimulate infection in plants (Powell, 1976; Warner & Mosse, 1980). However, little attention has been paid to external hyphae or vesicle components. This study examines whether the different components of mycorrhizal fungi have the ability to infect roots of Elaeis guineensis Jacq. (oil palm) and Theobroma cacao L.(cocoa),two important agricultural crops in Malaysia.

Materials and Methods

Pregerminated seedlings of E. guineensis and T. cacao were grown in garden soil and tin tailings sand which were fumigated with methyl bromide. Various components of the VA mycorrhizal fungi, Glomus fasciculatum (Thaxter) Gerd. & Trappe, Acaulospora scrobiculata Trappe and Gigaspora margarita Becker & Hall, were obtained from pot cultures of Zea mays L. and used as inoculum (Table 1). For spore inoculum, about 100 spores of G. fasciculatum, 30 of A. scrobiculata or 50 of G. margarita were used per plant. Control plants received combined leachings of the fungi. The plants were fertilized once with half-strength Hoagland's solution and then weekly with nutrient solution minus phosphorus. After 20 and 45 days the roots were examined for VA mycorrhizal infection.

Results and Discussion

The development of VA mycorrhizal varied with the fungal species and the type of inoculum component ((Table 1). Very little or no infection occurred after 20 days but by 45 days it was significant. Soil inoculum was more rapidly infective than the other components. This is probably because of mixed components of infective propagules.

Infections developed more quickly from root pieces of G. fasciculatum and A. scrobiculata than from spores. The delay observed in spore infection may be due to spore age or the fact that spores produce a pre-infection phase (Powell, 1976). Biermann and Linderman (1983) found that intraradical, but not extraradical, vesicles could act as propagules. However in this study, although G. fasciculatum and A. scrobiculata form intraradical vesicles, only those of G. fasciculatum were observed to be infective. It may be because vesicles of G. fasciculatum in roots often become thick-walled and function as spores (Gerdemann & Trappe, 1974). For G. margarita, the spores were more infective than mycorrhizal roots and the extraradical vesicles were not infective. Powell (1976) suggested that the infective patterns of VA mycorrhizal fungi are probably related to different nutrient reserves in the specific inoculum component.

External hyphae infected the two hosts in varying degrees thus emphasising the possibility of some saprophytic growth of hyphae in soil (Warner & Mosse, 1980). The ability of external hyphae and mycorrhizal roots to survive and act as propagules may partly explain the high levels of infection and low spore numbers reported earlier in some Malaysian soils (Nadarajah, Nawawi & Stone, 1981).

Table 1: Colonization of hosts after 20 and 45 days with components of inoculum from species of VA mycorrhizal fungi

a) Host: Elaeis guineensis

Inoculum component (amount)	% infection with fungi[1]					
	Gf		Ac		Gm	
	20d	45d	20d	45d	20d	45d
control	0	0	0	0	0	0
Soil (1g)	6	32	9	29	8	20
spores	2	20	0	17	10	20
roots (0.3g)	6	30	7	29	2	10
vesicles (100)	0	12	0	0	0	0
external hyphae (0.1g)	2	17	0	11	0	11

b) Host: Theobroma cacao

Inoculum component (amount)	% infection with fungi[1]					
	Gf		Ac		Gm	
	20d	45d	20d	45d	20d	45d
control	0	0	0	0	0	0
soil (1g)	8	38	9	34	11	30
spores	0	21	2	20	8	34
roots (0.3g)	6	30	9	36	2	12
vesicles (100)	0	10	0	0	0	0
external hyphae (0.1g)	0	12	4	19	0	15

[1] Gf=G. fasciculatus, Ac=A. scrobiculata, Gm = G. margarita

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*Research grant from International Foundation of Science, Sweden, is acknowledged.

EVALUATION OF VA-MYCORRHIZA AS A PARAMETER IN BREEDING FIELD-PEAS.

By

Anni Jensen

Keywords Pisum sativum, plant height, root length

Introduction

The aim of this work was to evaluate VA-mycorrhiza (VAM) and also root length as parameters in breeding field peas in order to develop pea varieties , that need less phosphorus fertilizer and are more drought tolerant. The variability among 398 pea varieties in root length, root dry weight, and VAM development in relation to plant height and top dry weight was investigated.

Methods and Materials

38 coloured flowered, 148 white flowered and 212 garden pea varieties from different countries were grown in a sandy soil with 42 ppm $NaHCO_3$-P in 30 cm PVC containers. All containers were burried in the field. VAM was established by naturally occurring fungi. Plants were harvested shortly before completely ripe. Root length and % VAM infection were assessed using a line intersection method.

Results and Discussion

For each recorded parameter the varieties are divided into 9 groups. In generel the coloured flowered peas had the best developed root system, the most intense VAM infection, the highest top dry weight and plant height. The garden peas were more sparse developed in the above mentioned characters and the white flowered peas in between. Figur 1 shows the percent varieties of the 3 pea types in the different groups of VAM infected root length.

The correlation coefficients between root length and plant height were low. This indicates that a well developed root system only to a limited extend is linked to tall plants, which means, that breeding a variety with well developed roots and a suitable plant height should be possible. Fig. 2 a and b shows the "correlation" between root length and VAM root length for respectively white flowered and coloured flowered peas. It is of interest to find the varieties in which VAM infection is able to keep up with a vigorous root growth.

In relation to developing pea varieties with dense root system and well developed VAM, breeding material seems to be offered in the following tested varieties: Lotta, Vreta, Marrowfat, NZ-361, Huka, Lenca, Marathon, Marma, Minerva, EFB 33 Pisello, Assas, Livia, Gali, Perdro, Minor, Bondi and Poneka.

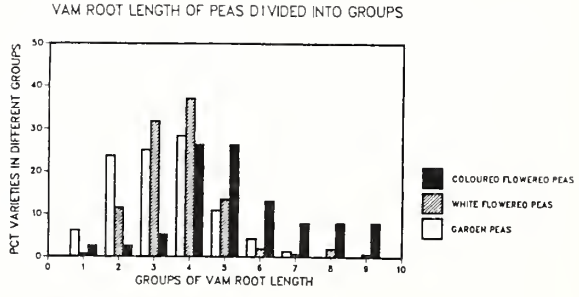

Figure 1.

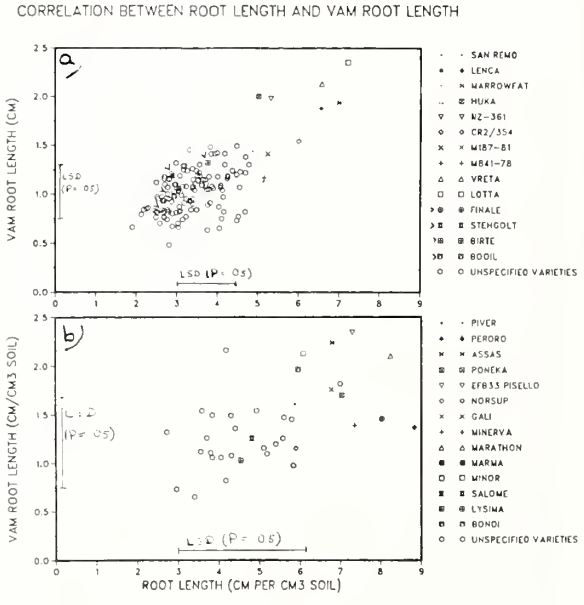

Figure 2.

MYCORRHIZAL RESPONSIVENESS OF FOUR CEDAR AND REDWOOD SPECIES OF WESTERN NORTH AMERICA

By

J. L. Kough, Randy Molina, and R. G. Linderman

Introduction

Commercial production of tree seedlings routinely includes a biocidal treatment of the soil to eliminate plant pests. These treatments may also reduce populations of beneficial organisms such as vesicular-arbuscular mycorrhizal (VAM) fungi which are very sensitive to these biocides. If plant species contingent upon mycorrhizal symbiosis for nutrient absorption are planted in treated soil, subsequent growth may be reduced or uneven in spite of apparently adequate soil nutrient levels. Arboreal species with thick magnolioid-type roots are especially susceptible to nutrient deficiencies in treated soil and respond with improved growth when inoculated with VAM fungi.

While VAM have been described in many Northwest conifer species, a determination of growth response over time with VAM fungi has not been done. This study examined responsiveness of Cuppressaceae and Taxodiaceae (Sequoia sempervirens-SS; Sequoiadendron giganteum-SG; Thuja plicata-TP; Calocedrus decurrens-CD) inoculated with three VAM fungi (Glomus deserticola-GD; G. epigaeum-GE; Acaulospora trappei-AT) maintained at two phosphate levels [1/4 strength phosphate (11 ppm) Long Ashton's Nutrient Solution (LANS) and full strength phosphate (44 ppm) LANS].

Materials and Methods

Seeds of the four tree species were stratified in moist paper towelling at 4C for three weeks prior to sowing on a flat of pasteurized sand:peat:soil (1:1:1). Seedlings with fully expanded cotyledons were transplanted into plastic growth tubes with approximately 160 cm^3 of pasteurized sand:soil (1:1) mixture. About 5 cm below the soil surface a 20 ml layer of colonized roots, VAM spores and sand from asparagus pot cultures was added. In addition, each VAM treatment and control received a 1 ml aliquot of a combined filtrate of all the VAM treatment to standardize the microflora between treatments. Plants were maintained in a glasshouse under supplemental sodium vapor lighting, watered daily and fertilized biweekly with 10 ml of either 1/4 strength phosphate (11 ppm) LANS or full strength phosphate (43 ppm) LANS. At 180 and 320 days after transplanting, the trees were measured and weighed. The root systems of ten seedlings were sampled and these root pieces were cleared and stained, and then assessed for VAM root length colonization. The foliage was dried and weighed, then analyzed for mineral content. The data were analyzed separately for each harvest using an analysis of variance to determine significant differences among treatments. Mean separation was done by the Tukey's test.

Results and Discussion

At 60 days after inoculation, VAM inoculated plants had developed true foliage faster than uninoculated controls irrespective of phosphate fertility. (TP: VAM-92% emerged vs. controls-35%; SS: VAM-89% emerged vs. controls-30%;CD: VAM-87% emerged vs. controls-57%; SG: VAM-91% emerged vs. controls-51%.) VAM-inoculated seedlings maintained with 1/4 strength phosphate (11 ppm) LANS were uniformly larger than uninoculated controls with 1/4 strength phosphate (11 ppm) LANS and larger or not significantly different from uninoculated controls maintained with full strength phosphate (43 ppm) LANS. VAM inoculated plants fertilized with full strength phosphate LANS were larger or comparable to uninoculated plants. No growth reduction was found with VAM at the higher level of phosphate fertility.

Growth enhancement from mycorrhizal colonization decreased with increasing seedling age. VAM responsive hosts may benefit in growth by mycorrhizal colonization mostly as young seedlings. Phosphorus input in seedlings is limited to seed reserves or that available in the small soil volume explored by the root radicle. Species with large seeds produce more initial root mass, withdraw more soil phosphorus and therefore may be less responsive to VAM colonization as young seedlings. Calocedrus decurrens, the species with the largest seeds in this study, did not respond to VAM at early harvests as much as other species but did show a growth response at later harvests.

The decreasing response to VAM colonization with increasing seedling age could be due to restriction of root growth in the VAM plants with limited volume of soil. Another possibility is a difference in root physiology. S. giganteum does not respond to VAM at the higher phosphorus fertility. This different root physiology may reflect ecological adaptations of S. giganteum which occurs on drier sites than the other species although it coexists with C. decurrens.

Additional benefits to commercial nurseries from VAM colonization of their stock are the enhanced transplant survival of VAM colonized stock and increased uniformity of the plants. VAM seedlings are significantly larger and more uniform as indicated by a lower coefficient of variability. This translates into a greater proportion of acceptable seedlings, thereby reducing culling loss.

Overall this study indicates that host plant physiology is the predominant determinant of the plant response to VAM colonization. Differences in plant growth response for the four tree species do not relate to VAM colonization alone given similar levels of root colonization (% root length VAM ranged from 16 to 40) among the different species at either harvest, with the same species of fungal symbiont and the same soil system. These results also emphasize the need to insure adequate soil volumes and include multiple harvests over time to be able to monitor the changes in host response that occur at different stages of plant development.

RESPONSE OF DIFFERENT GROWTH STAGES OF VIGNA UNGUICULATA (CV. 58-185) TO INOCULATION WITH GLOMUS MOSSEAE

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Response of Vigna unguiculata (CV. 58-185) to inoculation with Glomus mosseae was estimated by periodic measurements of shoot dry weight, nodule dry weight, N_2 fixation (C_2H_2 reduction activity), N and P content during the growth of the plants.

Shoot dry weight of inoculated plants was significantly different from that of non inoculated plants only 45 days after planting.

Dry weight of nodules and N_2 fixation (C_2H_2 reduction activity) of inoculated plants were found to be significantly higher than those of non inoculated plants as early as the 20^{th} day after planting although there was no significant difference in N content (%).

Shoot P content (%) of inoculated plants and non inoculated plants decreased progressively during the first 20 days, P content of inoculated plants being lower than of non inoculated ones. Then, P content of inoculated plants rapidly increased whereas P content of non inoculated ones remained constant.

Increase of dry weight of nodules, N_2 fixation and P content of inoculated plants corresponded to the onset of the development of the external hyphae of Glomus mosseae on mycorrhizal roots.

AGRONOMIC RESPONSE OF MYCORRHIZAL SOYBEAN TO DROUGHT STRESS

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A greenhouse study was conducted to evaluate the ability of the fungal symbiont, Glomus fasciculatum, to improve growth and yield response of drought-stressed soybeans [Glycine max (L.) Merrill]. Soybeans were grown in a high P soil, typical of the eastern Nebraska region. Factorial treatments, including the presence and absence of G. fasciculatum and drought stress, were arranged in a randomized, complete block design with twelve replications. Water was withheld from stress treatments for 9 days during the pod elongation stage, and two harvest dates were included: immediately following the stress period and at physiological maturity. Glomus fasciculatum infection had a greater relative effect on seed yield of drought-stressed compared to nonstressed plants, indicating a direct relationship between enhanced drought tolerance and increased yield of VAM-infected soybeans. Increased yield was attributed to a reduction in pod abortion. Drought stress reduced shoot dry weight, P content, predawn leaf water potential, and G. fasciculatum infection rate of plants harvested immediately following the stress period. Enhanced drought tolerance of infected plants, noted by a reduced root-to-shoot ratio, was attributed partly to improved host P nutrition. Total P uptake of stressed plants was significantly higher for infected plants. Although differences in predawn leaf potential between infected and noninfected, stressed plants were detected, the variation was small, suggesting root extraction of soil water was only slightly enhanced by G. fasciculatum. The results of this study verify the potential of G. fasciculatum to improve soybean yield under drought conditions.

INDIGENOUS MYCORRHIZAL BLACK WALNUT LARGER AFTER TWO YEARS

By

Felix Ponder, Jr.

Keywords--Juglans nigra, Glomus fasciculatus, field performance, bare-root seedlings, container-grown seedlings

Introduction

Black walnut (Juglans nigra L.) is known to be endomycorrhizal. Some growth differences attributed to type of mycorrhizae have been shown in greenhouse and nursery studies (Fardelmann 1981, Ponder 1979, Schultz et al. 1981), but little information is available on the possible field performance of mycorrhizal walnut seedlings. The standard root pruning practices for producing bare-root planting stock may reduce the number of mycorrhizal inoculated rootlets as well as reduce the number of live, fine roots from which most root initials originate. Container-grown planting stock should have more fine roots because no root pruning is necessary. The purpose of this study was to compare the growth of bare-root nursery seedlings with that of container-grown black walnut inoculated with either Glomus fasciculatus L. or indigenous mycorrhiza.

Methods and Materials

One-fourth liter of either soil from the planting site or G. fasciculatus soil-vegetative inoculum (containing Arachis hypogaea L. roots) was mixed with a 1:1 (v/v) mixture of methyl bromide fumigated sand and peat in each of 200 1.1 l factory-fresh milk containers. Black walnut seeds collected the previous year from a single tree in southern Illinois were surface sterilized with 10% (v/v) laundry bleach, pregerminated in plastic bags, and planted in containers in a shaded greenhouse. Seedlings were fertilized at 4-week intervals with 5 g of 12-12-12 commercial fertilizer and watered with distilled water as needed. Seedlings were placed in a shade house in October, and later covered with plastic overlain with wheat straw for overwintering. Conventionally grown 1-year-old bare-root seedlings were purchased from the Vallonia State Nursery in Indiana and the West Virginia DNR nursery and were delivered at the requested time for spring planting.

Soil at the planting site is moderately well drained and moderately deep with a dense firm layer at 66 cm. The mean soil nutrient contents in the topsoil were 45 ppm NO_3-N, 72 ppm P, 180 ppm K, 1,120 ppm Ca, and 96 ppm Mg. Mean soil pH was 5.6. To prepare the site, the well-developed Festuca arundinacea Schreb. sod was plowed twice and rototilled in the fall when the fescue began to recover. The following spring, seedlings were planted in a randomized complete block design with 4 blocks and 4 treatments per block of 25 seedlings each spaced 1.2 m apart. Roots from 25 unplanted seedlings from each treatment were examined for mycorrhizal infection. Pre- and post-emergent herbicides were applied annually to control weeds.

Results and Discussion

Two growing seasons after outplanting, seedlings inoculated with soil from the planting site were significantly (P=0.05) taller and larger in diameter than seedlings inoculated with G. fasciculatus or bare root seedlings (table 1). Total growth of indigenous inoculated seedlings exceeded other treatments by more than 100 percent in height and 50 percent in diameter. Examination of randomly selected seedlings at time of planting showed all to be endomycorrhizal. However, except for G. fasciculatus, the symbionts responsible for the infection were not identified. Apparently seedlings growing in the indigenous soil contain a mycorrhizal population compatible with local soil conditions and other microorganisms of the planting site, while the introduced microflora may be subjected to soil environmental conditions that greatly restrict its efficiency.

Typical for black walnut, the effect of transplanting was less for container-grown than for bare-root seedlings as evidenced by better first season growth. For the period, height and diameter growth of container-grown seedlings exceeded bare-root seedlings by 354 and 47 percent, respectively.

These results are another example of the variation of the symbiotic value of mycorrhizal fungi (Fardelmann 1981). It is important to test mycorrhizal symbionts for growth enhancement, but the most efficient symbionts may be those already in the microflora of the planting site.

Table 1. Average height and diameter growth of outplanted mycorrhizal black walnut following different inoculation and seedling production environment.[1/]

Treatments	After planting 1982 Height (cm)	After planting 1982 Diam (mm)	Fall 1983 Height (cm)	Fall 1983 Diam (mm)
Indigenous	28.2 c	5.8 b	82.5 a	15.3 a
Indiana	51.1 a	7.0 a	74.4 b	13.5 b
W. Virginia	43.4 ab	6.6 a	70.6 bc	13.1 b
G. fasciculatus	39.5 b	5.8 b	63.3 c	11.9 c

[1/] Means within a column not sharing a common letter differ significantly (P=0.05) by Duncan's multiple range test.

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ECOLOGY

NITROGEN-FIXING BACTERIA ISOLATED FROM WITHIN SPOROCARPS OF THREE ECTOMYCORRHIZAL FUNGI

by

C. Y. Li and Michael A. Castellano

Keywords--Klebsiella, Pseudomonas, Hymenogaster, Tuber, Suillus, bacteria, nitrogen

INTRODUCTION

Fungi utilize large amounts of nitrogen in the production of sporocarps and spores and it has been suggested that fungi can partially satisfy this demand through fixation of atmospheric nitrogen by associated N-fixing bacteria (Larsen et al. 1978).

Larsen et al. (1978) showed that sporocarps of Fomitopsis pinicola (Swartz:Fr.) Karst., Fomes fomentarius (L.:Fr.) Kick., and Echinodontium tinctorium (Ell. & Ev.) Ell. & Ev. had nitrogenase activity as demonstrated by the acetylene-reduction technique.

In this study we examined the nitrogenase activities of N_2-fixing bacteria isolated from interior sporocarp tissue of three ectomycorrhizal fungi.

MATERIALS AND METHODS

Fresh sporocarps of Hymenogaster parksii Zeller & Dodge, Suillus ponderosus Smith and Theirs, and Tuber melanosporum Vitt. were collected and rinsed with tap water, then immersed in 70% alcohol and sonicated for 5 minutes, followed by immersion in 2% sodium hypochlorite for 5 minutes and rinsed in two changes of sterile distilled water. Sporocarps were aseptically split open, and interior tissue was removed and macerated in a mortar and pestle with distilled water (1:5 v/v). Bacterial cultures were made by streaking or by serial dilution on nitrogen-free media (Burk 1930).

Sporocarp extracts were prepared then 0.1 ml was added to 20 ml liquid media. An aqueous bacterial suspension (0.05 ml) was inoculated into 60-ml serum bottles which contained 20 ml of nitrogen-free media. Bottles were incubated under aerobic or microaerophilic conditions (99% nitrogen, 1% oxygen) for 2 days at 30 °C. Acetylene was injected into each bottle to 10% (v/v); the bottles were gently swirled immediately after addition of acetylene and left to stand at 30°C. After 1, 3, 5, 7, 9, and 24 hr, 0.05-ml gaseous samples from each bottle were removed and analyzed for ethylene and acetylene with a gas chromatograph.

RESULTS

Three species of N_2-fixing bacteria were isolated in nitrogen-free pure culture, one each from interior sporocarp tissue of Hymenogaster parksii, Suillus ponderosus, and Tuber melanosporum. All bacterial isolates required sporocarp extract and microaerophilic conditions for N_2 fixation as demonstrated by the acetylene reduction method. Without sporocarp extract, the bacteria neither grew nor reduced acetylene under aerobic or microaerophilic conditions. Bacteria isolated from H. parksii and S. ponderosus (Pseudomonas fluorescens Migula biotype 3 and biotype 2, respectively) were gram-negative rods, but they differed in color of colony on nutrient agar. The bacterium isolated from T. melanosporum (Klebsiella pneumoniae (Schroeter) Trevisan) was gram-variable pleomorphic cells, with straight to curved rod or coccoid morphology. Sporocarp extract of H. parksii and T. melanosporum was equally effective in stimulating acetylene reduction of all bacterial isolates. After 24 hours, the K. pneumoniae isolate reduced significantly more acetylene than did either P. fluorescens isolate.

DISCUSSION

Klebsiella pneumoniae and other N_2-fixing bacteria are found associated with many decay fungi. Larsen et al. (1978) suggest that development of fungal sporocarps may require an extramural source of nitrogen, possibly supplied by associated N_2-fixing bacteria. Evans et al. (1972) reported a stimulatory effect of soybean nodule extract on growth and nitrogenase activity of bacteria isolated from the surface of nodules. Apparently sporocarp extract also provided a growth factor required by the bacteria for growth and subsequent nitrogenase activity. Within a sporocarp near anaerobic growth conditions can exist which are requisite to growth of these bacteria.

Fungal hyphae serve as migration pathways and growth regions for P. fluorescens in soil (Arora et al. 1983); P. fluorescens also is reportedly involved in suppression of fungal root diseases in soil (Scher and Baker 1982).

These findings are an exciting and hitherto unrecognized source of nitrogen fixation in western forests that may represent a substantial input of N into the ecosystem, especially during the autumn and spring fruiting seasons.

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SUCCESSION OF ECTOMYCORRHIZAL FUNGI ASSOCIATED WITH EUCALYPTS ON REHABILITATED BAUXITE MINES IN SOUTH WESTERN AUSTRALIA.

By

J.H. Gardner and N. Malajczuk

Keywords--*Eucalyptus*, ectomycorrhizal sporocarps

Introduction

Bauxite mining in the south-west of Western Australia involves forest clearing followed by open cut mining. Each year some 400 ha of jarrah (*Eucalyptus marginata*) forest is cleared, and after mining, is rehabilitated with a new eucalypt forest designed to replace the functions of the original forest. In the rehabilitation process the original 40 cm of surface soil is replaced on to a clay subsoil; the soil profile having been truncated by removel of 3 m of bauxite. There is prior deep ripping of the clay surface to enhance root penetration and fertilization with N and P to replace some of the nutrients lost in clearing and mining (Tacey 1979).

Ectomycorrhizae are important in tree growth, and particularly nutrient uptake (Harley and Smith 1983), but little is known about mycorrhizal fungi associated with eucalypts in the original forest or on rehabilitated mine sites. We examined the ectomycorrhizal fungal population from a series of even age eucalypt stands on rehabilitated sites and compared these with fungi associated with surrounding jarrah forest.

Methods and Materials

Putative ectomycorrhizal sporocarps were collected in 1983 at c fortnightly intervals from around (2 m radius) the bases of five eucalypt species (*E. marginata, E. resinifera, E. calophylla, E. wandoo* and *E. maculata*) on the mine sites. Similar collections were carried out from the adjoining, undisturbed jarrah forest in areas (5 x 5 m) around selected trees. All collections were differentiated into species and/or genera and accurately mapped to determine spatial distribution.

Results and Discussion

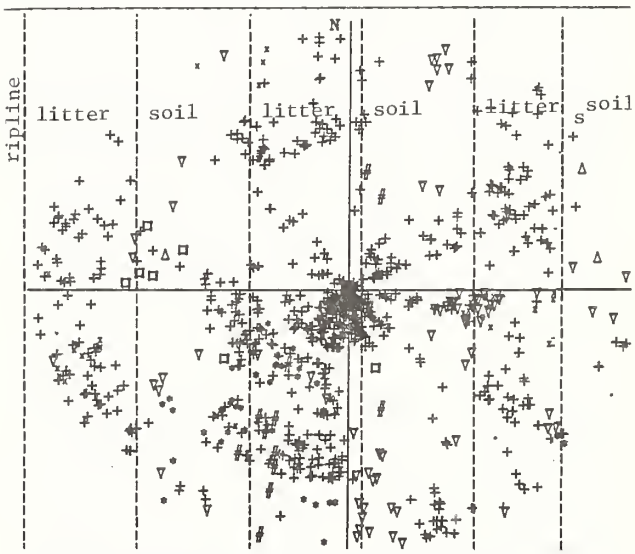
The diversity of putative ectomycorrhizal fungi increased with increasing age of eucalypt plantings. After 7 yrs., the number of ectomycorrhizal fungi species on the mine sites represented less than half of these found in adjoining, undisturbed jarrah forest (Table 1). Of particular significance was the marked succession in the fungal genera with tree age; *Pisolithus, Scleroderma, Laccaria* representing the early-colonizing fungi and *Ramaria, Cortinarius, Paxillus* and *Russula* species as the late colonizers (Table 1). In most instances the fungi showed distinct patterns of growth around trees and the occurrence of particular spp. was invariably correlated with the presence of litter development beneath the trees. This was particularly highlighted on the mine sites where litter development in the troughs of riplines encouraged the fruiting of *Cortinarius, Paxillus* and *Ramaria* species while on the crest of these troughs, *Laccaria* and *Scleroderma* species predominated (Figure 1).

Preliminary examination of the eucalypt root systems indicated a close relationship between the fungal species fruiting on the mined sites and the development of specific ectomycorrhizal root structure characteristics of these groups.

These results support the general concept proposed by Mason *et al.* (1983) that ectomycorrhizal fungi follow a successional development with tree age and it would appear that one of the important factors influencing these changes in fungal genera is litter build-up. More importantly, studies of this nature provide useful information on the choice of suitable fungi for inoculation of eucalypt seedlings.

Table 1. Major putative ectomycorrhizal genera and number of species associated with eucalypts on mine sites and in the jarrah forest.

Age of Rehabilitation (yrs)	Ectomycorrhizal fungi Major genera	Number of Species
1	*Pisolithus, Sclero-derma, Laccaria*	4
3	*Laccaria, Scleroderma*	9
7	*Amanita, Cortinarius, Paxillus*	18
Jarrah forest c 100	*Cortinarius, Paxillus, Ramaria, Russula*	50



KEY + *Cortinarius sanguineus* ∇ *Laccaria laccata* □ *Lycoperoon gracilis* Δ *Pisolithus tinctorius* x *Paxillus muelleri*

Figure 1. Annual spatial pattern of sporocarps associated with *E. resinifera* (7 yr old) showing species occurrence on litter or soil.

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ECTOMYCORRHIZAL DEVELOPMENT OF JACK PINE SEEDLINGS BY INTRODUCED AND INDIGENOUS FUNGI

By R.M. Danielson, S. Visser and D. Parkinson

Keywords--E-strain, Hebeloma, Thelephora, Laccaria, Cenococcum, Pisolithus, Astraeus

Introduction

Extensive areas of Alberta are underlain with deposits of oil sands suitable for surface mining. The residuals of mining are overburden and oil sand tailings. The latter are nearly pure fine-grained sands. Current reclamation procedures improve the properties of the tailings by the addition of muskeg peat and clayey overburden. As reconstructed soils are nutrient deficient and contain small amounts of mycorrhizal inoculum a study was initiated to determine (1) the effects of inoculation on the field performance of jack pine, (2) the persistence of introduced fungi and (3) the contribution of indigenous fungi to the mycorrhizae of seedlings.

Methods and Materials

Container-grown jack pine were inoculated with 12 mycorrhizal fungi (peat-vermiculite-MMN). Ten seedlings from each inoculation treatment were sampled and the conversion of short roots (300/plant sampled) to mycorrhizae determined. Seedlings were planted on a routine site on a tailings pond dyke. Ten seedlings from each treatment were sampled after 1 and 2 growing seasons to determine plant growth and mycorrhizal development. Only roots extending from planting plugs were assayed for mycorrhizae.

Results

When planted there were no significant differences in seedling size among treatments. Inoculation was successful with 9 of 12 fungi (Table 1). After 1 growing season, inoculation had no effect on growth or seedling survival. Indigenous fungi formed mycorrhizae on 4% of the short roots and 31% of the seedlings. Four of 9 introduced fungi were successful colonizers of roots in the reconstructed soil.

After 2 growing seasons, seedlings inoculated with E-strain and Thelephora terrestris were significantly larger than uninoculated seedlings. Only 3 fungi, E-strain, T. terrestris and Hebeloma sp., formed a significant quantity of mycorrhizae. The other fungi were absent or only present on older roots.

Indigenous fungi formed mycorrhizae on 33% of the short roots by the end of the second year. Of the 16 species detected, E-strain fungi were common and the most aggressive. A cystidial ascomycete, the I-type, was common but less aggressive than E-strain. Mycelium radicis atrovirens occurred on the largest number of seedlings but was nonaggressive. Two unknown basidiomycetes formed mycorrhizae on 5% of the short roots and other basidiomycetes formed only a minor portion of the mycorrhizae. A Rhizopogon-like fungus occurred on a large number of seedlings but was nonaggressive, resulting in patchy infection patterns.

Table 1. Ectomycorrhizae of jack pine seedlings formed by introduced fungi.

Inoculation treatment	Length of time in field		
	Preplant	One season	Two seasons
	% ectomycorrhizae		
E-strain	91	93	82
Hebeloma sp.	99	48	44
Thelephora terrestris	100	75	52
Laccaria proxima	99	42	0
Cenococcum geophilum	57	2	12
Pisolithus tinctorius	54	6	6
Astraeus hygrometricus	48	4	7
Lactarius paradoxus	32	1	0
Sphaerosporella brunnea	17	0	0
Amphinema byssoides	0	0	0
Hydnum imbricatum	0	0	0
Tricholoma flavovirens	0	0	0
Control	0	0	0

Conclusions

1. Amphinema byssoides, Hydnum imbricatum and Tricholoma flavovirens failed to form mycorrhizae with container-grown seedlings. These fungi are multi- or late successional stage fungi in natural jack pine stands.

2. Six of the 9 fungi introduced failed to colonize significant portions of the roots that grew from the planting plug. This failure was not due to competition from other mycorrhizal fungi.

3. The major fungi indigenous to the peat-tailing sand mixture and compatible with jack pine were ascomycetes. These fungi were slow to colonize seedlings and varied considerably in aggressiveness.

4. Plant performance was significantly improved by inoculation with E-strain and Thelephora terrestris. Of the fungi tested, these two were the best adapted to the soil encountered by emerging roots.

Acknowledgements

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FUNGI IN THE MYCORRHIZOSPHERE OF BLACK SPRUCE IN BOREAL CANADA

By

Richard C. Summerbell

Keywords--Picea mariana, Laccaria laccata, competition, rhizosphere, Trichoderma

Introduction

The potential effect of soil microorganisms on mycorrhiza-formation is a subject about which little is known. Bowen and Theodorou (1979) have shown that certain soil bacteria may enhance or inhibit the colonization of roots by ectomycorrhizal fungi, but few parallel effects have been demonstrated using soil fungi. In the study presented here, populations of filamentous fungi associated with ectomycorrhizal roots of black spruce were enumerated. Characteristic inhabitants of the mycorrhizosphere were tested for their effects on mycorrhiza-formation in vitro.

Materials and methods

Black spruce roots from a number of boreal forest sites were collected and serially washed in 20 changes of sterile water. Some were also surface-sterilized in 100 ppm. mercuric chloride. Mycorrhizal root tips and fragments of proximal root bark were plated onto isolation media. Water from the washing procedure was plated out onto media restrictive of fungal colony size. For comparison, similar procedures were carried out with roots of Cornus canadensis, an endomycorrhizal understory plant.

In in vitro studies, spruce trees were grown axenically in closed flasks containing a peat/vermiculite mixture amended with a mineral nutrient solution. After leaves had emerged, flasks were inoculated with mycelium of Laccaria laccata, an ectomycorrhizal basidiomycete, and with mycelium of a selected mycorrhizosphere inhabitant. Controls were inoculated with L. laccata alone, or with mycorrhizosphere fungus alone. Trees were harvested after six weeks at 18°C. (18 h. light, 6 h. dark) and examined for the presence of mycorrhizae. Where appropriate, the proportion of mycorrhizal root tips was estimated with a random sampling procedure.

Results and Discussion

The fungal species most commonly isolated from washed spruce mycorrhizae are shown in Table 1. A similar assemblage of isolates was obtained from washed fine roots of C. canadensis. Surface-sterilized spruce roots yielded Mycelium radicis atrovirens and "sterile white 1" almost exclusively, while root bark and root washings mainly gave rise to colonies of sporulating Hyphomycetes and Zygomycetes. The results suggest that fungal populations associated with surfaces of ectomycorrhizal spruce root tips are similar to populations on other root surfaces in the boreal forest. Such populations may differ from populations found in adjacent soils, including rhizosphere soils.

Table 1. Common fungal species isolated from washed mycorrhizae of Picea mariana. 1/

Fungus sp.	% of total isolates
Mycelium radicis atrovirens	14.4
"Sterile white 1"	13.6
Micromucor isabellina	10.6
Penicillium spinulosum	8.1
Trichoderma viride	4.7
Trichoderma polysporum	4.2
"Sterile dark 1"	3.4
Mortierella verticillata	3.4
Fusarium proliferatum	2.9

1/ Preliminary results; 247 isolates, 54 spp.

In in vitro trials, L. laccata colonized 100% of susceptible root tips in the absence of other fungi, as well as in the presence of five mycorrhizosphere species (Fusarium proliferatum, "sterile white 1", Mortierella verticillata, Micromucor isabellina, Penicillium spinulosum). Three species caused an inhibition of mycorrhiza-formation: two Trichoderma species, and Trichosporon beigelii (Table 2). The nature of the inhibition brought about by the latter fungus is unknown, but Trichoderma species are well known as aggressive opportunistic mycoparasites. To my knowledge, an inhibition of mycorrhiza-formation by these fungi has not previously been demonstrated.

Table 2. Formation of Laccaria laccata mycorrhizae in the presence of selected mycorrhizosphere fungi.

Mycorrhizosphere fungus	# of root tips counted	% mycorrhizal
Trichoderma polysporum	448	20.1
T. viride	300	nil
Trichosporon beigelii	292	67.1
control	200	100

N.B.: counts do not include primary long root tips.

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SUBARCTIC TRUFFLES: SPECIATION, RANGE EXTENSION, ASSOCIATIONS AND SPORE VECTORS

By

G.A. Laursen, R.A. Mowrey and J.F. Ammirati

Keywords--Alpova diplophloeus, Gautieria graveolens, G. otthii, Hysterangium separabile, Leucophleps spinaspora, Elaphomyces muricatus, Geopora cooperi f. cooperi

Introduction

It has long been suspected and more recently documented for several interior Alaskan forest types that hypogeous false and true truffle fungi play an important role in forming mycorrhizal relationships with the larger and frequently more economically important vascular plant species such as white spruce (Picea glauca (Moench) Voss), paper birch (Betula papyrifera var. humilus (Reg.) Fern. and Raup), balsam poplar (Populus balsamifera L.), quaking aspen (Populus tremuloides Michx.) and tamarack (Larix laricina (Du Roi) K. Koch). The presence of mycorrhizal fungi with less important species such as black spruce (Picea mariana (Mill.) B.S.P.) are documented and suspected to occur with a host of species; i.e., alder, poplar and willow of riparian habitats.

Hypogeous fungi in white and black spruce ecosystems have been collected and examined from excavating areas containing pits freshly dug by small mammals. The northern red squirrel (Tamiasciurus hudsonicus), northern flying squirrel (Glaucomys sabrinus), and northern red-backed vole (Clethrionomys rutilus) have been shown through gut analyses to be important mycophagists and spore vectors for truffle fungi in interior Alaska. In fact, truffle spore dispersal is accomplished almost totally by way of small mammal mycophogy. During peak (late summer-early fall) fruiting, it has been shown that hypogeous fungi may comprise 100 percent of the diets of small rodents restricted to plant communities dominated by the above listed woody plant species. Fecal deposition insures widespread dispersal of spores (Figure 1) often needing alimentary canal treatments before they will germinate.

Figure 1. Spores (SEM) of hypogeous fungi as numerically identified under Results and Discussion, X5000.

Methods and Materials

More than 1,000 preserved small mammal specimens of seven species will be examined for occurrence of hypogeous fungi to identify those that function as important spore vectors in white spruce and black spruce ecosystems. When mature, distinctive fungal odors enable rodent location of the hypogeous fungi.

Fungi will be examined and identified with the use of a Leitz Dialux transmitting light microscope. Spores from sporocarps and stomach contents are being photographed with an SEM.

Results and Discussion

Together, ten false and true truffles (Figure 2) have thus far been identified from Alaska's interior; i.e., closed spruce-hardwood forests; open, low growing spruce forest; and shrub thickets. They are: 1) Alpova diplophloeus; 2) Gauteria graveolens (aff. morchellaeformis var. globospora); 3) G. otthii; 4) Hysterangium cistophilum; 5) H. separabile; and 6) Leucophleps spinaspora, the false truffles; whereas 7) Elaphomyces granulatus; 8) Geopora cooperi forma cooperi; 9) G.c. f. gilkeyae; and 10) Tuber cf. californicum are true truffles.

Other truffles fungi such as Gastroboletus Hydnotrya, 11) Hymenogaster, Martellia, Mycolevis, Rhozopogon, Sedecula, and Thaxterogaster have been tentatively identified from spores found in rodent gut contents.

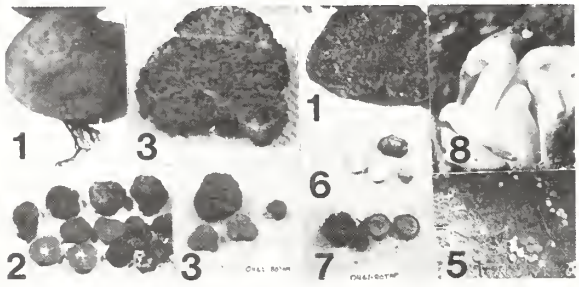

Figure 2. Sporocarps of hypogeous fungi identified numerically in Results and Discussion.

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NORTHERN FLYING SQUIRREL: THE MOONLIGHT TRUFFLER

By

Z. Maser, R. Mowrey, C. Maser, and W. Yun

Keywords--Mycophagy, northern flying squirrel, coniferous forests.

Introduction

The northern flying squirrel (Glaucomys sabrinus) is a common resident of coniferous and mixed conifer-hardwood forests of North America. Its range extends from the Arctic tree line throughout the northern coniferous forests of Alaska and Canada, south through the Cascade Range of Washington and Oregon and the Sierra Nevada almost to Mexico, the Rocky Mountains of Utah, and the Appalachian Mountains to Tennessee (Hall 1981). Although seldom seen because of its nocturnal habits, the flying squirrel is important in the dispersal of spores of hypogeous, ectomycorrhizal fungi (Fig. 1).

The study of mycophagy (fungal consumption by small mammals) in the northern flying squirrel is being conducted in Alaska. Maser et al. (in press) have studied their food habits in northeastern and northwestern Oregon. Another such study is underway in southwestern Oregon. Maser et al. (1981) and Mowrey and Zasada (in press) have also conducted studies on use of den trees, aggregation of individuals, and segregation of sexes in Oregon and Alaska, respectively.

Methods and Materials

Flying squirrels used in the study from Oregon were dead-trapped or shot. The squirrels from Alaska were live-trapped and only fecal samples obtained; occasionally one was found dead in a trap. Stomach contents and the terminal fecal pellets in the rectum were preserved separately in vials with 10 percent formalin.

Stomach contents and fecal pellets were microscopically examined at 40 and 100X. A small sample was placed on a microscope slide, a drop of Melzer's reagent added, and a cover slip put in place. Items were identified for each squirrel and recorded on cards. Percent volume of each food item was estimated and recorded.

Results and Discussion

The northern flying squirrel was found to eat a variety of items in Oregon (lichens, male conifer flowers, starchy material, and unidentified items), but hypogeous and epigeous fungi predominate (Maser et al., in press). Lichen (Alectoria fremonti) was the squirrels' predominant food in northeastern Oregon from December through June; hypogeous fungi then became the principal item. Of the 22 different kinds of food items encountered, 19 were hypogeous fungi (Table 1). Within the coniferous forests of Oregon, the flying squirrel is a fungal gourmet.

In Alaska, flying squirrels have been observed eating hypogeous (Table 1) and epigeous fungi, berries, tree lichens, newly flushed growth tips of spruce, and dried fungi cached in limbs at middens by red squirrels (Tamiasciurus hudsonicus) (Mowrey 1982). Research is currently underway on food habits, forest structure, and nests of the flying squirrel.

Table 1. Genera of hypogeous fungi found in the digestive tract and fecal pellets of the northern flying squirrel in Oregon and Alaska.

Fungal species		Fungal species	
Oregon	Class[1]	Alaska	Class[1]
Elaphomyces spp.	A	Elaphomyces sp.	A
Gautieria spp.	B	Gautieria sp.	B
Geopora sp.	A	Geopora sp.	A
Hymenogaster sp.	B	Hymenogaster sp.	B
Hysterangium sp.	B	Hysterangium spp.	B
Martellia sp. & relatives	B	Martellia sp. & relatives	B
Rhizopogon sp. & relatives	B	Rhizopogon sp.	B
Thaxterogaster sp.	B	Gastroboletus	B
Choiromyces sp.	A	Thaxterogaster sp.	B
Genabea sp.	A	Hydnotrya sp.	A
Genea sp.	A	Gautieria otthii	B
Leucogaster spp.	B		
Leucophleps sp.	B		
Melanogaster spp.	B		
Pezizales sp.	A		
Picoa sp.	A		
Radiigera sp.	B		
Tuber spp.	A		
Endogonaceae	Z		

[1]A Ascomycotina, B Basidiomycotina, Z Zygomycotina.

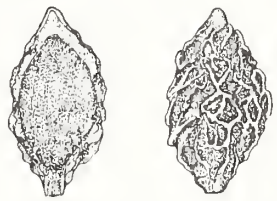

Figure 1. Spores of the hypogeous Hymenogaster parksii A. Cross section; B. Surface.

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ECOLOGICAL HABITAT OF MYCORRHIZAE OF NORTHERN TEMPERATE CLIMAX FORESTS

By

I. Girard and J.A. Fortin

Keywords: fir forest, maple forest, spruce forest.

Introduction

The different mycorrhizal associations are widely distributed among the vascular plants around the world. As intimate plant associates, mycorrhizae form a basic component of plant communities and as such, may reflect the environment of the community. This suggests that the types of mycorrhizae may be community - or environment - specific, or at least show preferences.

The goal of this report is (1) to characterize the mycorrhizal association of each plant species belonging to six stations representing five climax forest associations and (2) to define the habitat of the mycorrhizae in relation to the biophysical components encountered.

Material and Methods

Six old-growth forest associations were sampled: Sugar Maple-Bitternut Hickory, Sugar Maple-American Basswood, Sugar Maple-Yellow birch, Sugar Maple-American Beech, Balsam Fir-Paper birch and Black Spruce forests. These forests are representative (except the Sugar Maple-American Beech) of the five climax forest associations of southern Québec, Canada. They were distributed along a continuous soil, flora and climatic gradient. Thus, this ecological sequence goes from a biota with high species diversity, fertile brown soils and a mild climate to a poor habitat with a low species diversity, podsols and a harsh climate (Table 1).

Plant species were sampled all along the growing season and the root system assessed for mycorrhizal types [Ecto-, VA, ericoid, arbutoid and orchidaceous mycorrhizae] based on their respective morphology and anatomy.

Results and discussion

Most of the sampled plant species were mycorrhizal (93%). Only endomycorrhizae were present on ferns and herbaceous plants, while ectomycorrhizae were found only on woody plants. Although several woody plants had VA mycorrhizae.

The same plant species specific kind of mycorrhizae was found throughout different forest associations; however the intensity of root colonization varied. The VA mycorrhizae showed less colonization in spruce forests than in maple forests for herbaceous plants with annually renewed root systems (e.g. Maianthenum canadense). On the other hand, the perennial root systems of woody plants, like Sorbus americana, were heavily colonized by VA in the spruce forest. This may reflect the low infection potential prevalent in the soils of the spruce forest.

A gradient in the mycorrhization was observed along with those of all biophysical components analysed (Table 1). The VA mycorrhizae are largely dominant in the Maple-Hickory forest association and gradually decrease to become poorly represented in the spruce forest. Inversely, the ectomycorrhizae increase from the Maple-Hickory associations toward the Spruce forest associations. In the case of ericoid mycorrhizae, they were observed only in the coniferous forests and increased toward the northern boreal forest. From these observations a preferential habitat can be perceived for the different types of mycorrhizae.

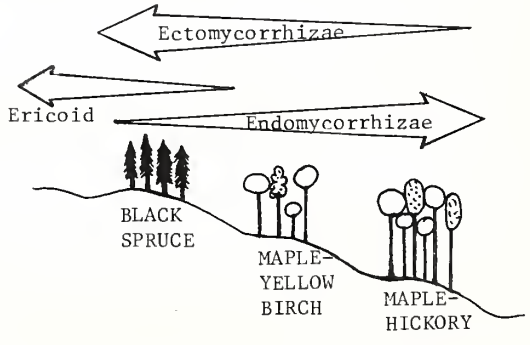
The VA mycorrhizae dominate in the decidous climax forests. They are associated with relatively fertile soils. The humus is a mull, the pH is slightly acidic, the nitrogen is available mainly as NO_3^- and the rate of turnover is relatively high. A high plant species diversity is present along with a mild climate.

The ectomycorrhizae dominate in the coniferous climax forests. They are well established on soils of low fertility. The humus is a mor, the pH is acidic, the nitrogen is available as NH_4^+ and the turnover is slow. A low number of plant species are found along with a harsh climate.

The ericoid mycorrhizae are associated with more difficult growth conditions than ectomycorrhizae. The soils are poor, very acidic and nitrogen is mostly tied and available mainly as organic molecules. Plant diversity is low and the climate is very harsh.

As a biophysical component, mycorrhizal associations vary along with the different environmental factors and communities. Mycorrhizae undoubtly show preferences toward a defined hatitat.

Table 1. Biophysical gradients in climax forests of southern Québec.



Humus	Mor	Moder	Mull
Soil	Ortstein Ferro-Humic Podsol	Dystric Brunisol	Melanic Brunisol
pH	2.9	4.2	5.8
C/N	37	26	14
Décomposition rate (years)	43.3	2.3	1.7
Earthworms	-	±	+
Plant species	16	76	100

Note: The soil data come from studies of several researchers of Laval University.

HYPOGEOUS, MYCORRHIZAL FUNGI ASSOCIATED WITH PONDEROSA PINE: SPOROCARP PHENOLOGY

By

Jack States

Keywords--Seasonal productivity, mycophagy, species abundance, Rhizopogon, Gautieria.

Introduction

Data presented represent a quantitative 4 yr study of hypogeous sporocarp production by putative mycorrhizal associates of Ponderosa pine (Pinus ponderosa Laws.). The study objectives were to evaluate species distribution and frequency within several pine stand types and to estimate relative abundance of these species on a seasonal basis. Methods of measuring sporocarp biomass were modified to identify areas of sporocarp concentration.

Methods and Materials

Monthly sporocarp production was measured in two stands, each with a mixture of two age-vigor classes, mature-yellow pine and young-blackjack pine.

Within six hectare plots, eight 50 m^2 sub-plots were intensively monitored for sporocarp production and constitute the basis of monthly production estimates. Sporocarp collection records were compared to rodent mycophagy records taken at the same time. Productivity estimates are based on mean dry weights of each species multiplied by the numbers of sporocarps produced yearly in one hectare. Relative frequency of species is calculated as the total number of occurrences in each sample (quadrat) as percent of the total samples taken.

Results and Discussion

A ranking of the genera according to percent frequency in fruitbody collections and compared to percent frequency in mycophagy records is presented in Table 1. Phenological data for the top six species collected are summarized in Table 2.

Table 1. Ranking of fungus occurrence based on frequency of genera in monthly mycophagy and sporocarp collections.

	Mycophagy Records (n = 490)		Sporocarp Collections (n = 391)	
Genus	Rank	Freq (%)	Rank	Freq (%)
Rhizopogon	RH	20.1	RH	39.0
Hysterangium	HY	13.2	HY	12.3
Gautieria	GA	12.9	GA	11.3
Sclerogaster	SC	11.2	SC	9.5
Geopora	GE	10.8	HM	8.9
Hymenogaster	HM	6.9	TU	5.4
		.9396*		

*Coefficient of rank correlation (p=.001).

Although sporocarps of Rhizopogon spp. outnumbered those of other genera, Gautieria is the most important contributor to total annual biomass. Rhizopogon spp. are infrequent during winter months but T. levissimum, S. xerophilum, H. separabile and H. parksii are constantly produced, even beneath a snowpack.

Table 2. Phenological data for hypogeous fungi associated with Ponderosa pine, data years 1979-1983.

Fungus species	Sporocarp $\bar{x}$ dry wt.(g)	Biomass %/yr	Fruiting season
Gautieria crispa	2.12	46.1	All year
Rhizopogon evadens	0.93	40.2	May-June
Hysterangium separabile	0.44	5.2	All year
R. subcaerulescens	0.36	3.9	June-Dec
R. ochraceorubens	1.46	0.9	Aug-Nov
Sclerogaster xerophilum	0.21	0.8	All year

Sporocarp distribution was found to be non-random and clustered beneath the more dense canopy of Young-blackjack pine stands than the mature-yellow pine. The greatest production of fruitbodies was found to be positively and significantly correlated with estimates of canopy cover (Table 3).

Table 3. Sporocarp distribution in random and selected quadrats of Ponderosa Pine. (Each sample type=16 quadrats=0.5 ha)

Sample type	No. stems (total)	Canopy Est. (mean*)	No. Sporocarps
Random sample points	196	3.13	363
Selected points for high canopy	860	4.13**	1285**
Selected points for low canopy	343	2.40	71

*Canopy estimate scale: 1 = no cover; 2 = low; 3 = moderate; 4 = high; 5 = total.
**Correlation coefficient for sporocarp versus canopy estimate = .952, regression p = .0127.

The differences in biomass estimates in kg/ha/yr using standard and selective techniques are illustrated in Table 4. Some stands of Ponderosa pine were found to be especially productive up to 22 kg/ha/yr. They occupied north facing slopes where water retention in the mineral soil was measurably greater. Studies are continuing to determine the effects of climate and host on sporocarp production.

Table 4. Sporocarp productivity estimates for major hypogeous species in pine stand using two sampling techniques.

Fungus Species	Site 1* kg/ha/yr	Site 2** kg/ha/yr
Rhizopogon spp.	1.36	2.69
Gautieria crispa	1.25	1.84
Hysterangium separabile	.13	1.54
Other species	2.42	1.75
Total	5.16	7.82

*Sample area of 16 quadrats, 50m apart = 0.5 ha
**Sample area as above but selectively placed in representative age classes with high canopy estimate.

CONSUMPTION OF HYPOGEOUS FUNGI BY THE DEER MOUSE (PEROMYSCUS MANICULATUS)

By

Gary A. Hunt and Zane Maser

Keywords--mycophagy, mycorrhizae, fungal biomass, spore dispersal.

Introduction

Obligate relationships between host trees, mycorrhizal fungi, and mammals have evolved in coniferous forest ecosystems. Although the relationship of mycorrhizal fungi and plants is well known, the role of mammals as dispersers of fungal spores has only recently been elucidated (Fogel and Trappe 1978, Maser et al. 1978). Study of stomach contents and fecal pellets shows that a diversity of small mammals consume hypogeous sporocarps and that defecated spores are viable as mycorrhizal inoculum (Trappe and Maser 1976).

Methods and Materials

The study was conducted in the Cascade Range east of Eugene, Oregon. During May and November 1983, fungi were collected in 14 stands varying in age and moisture status. Stand types and ages were: replanted clearcut, 5 years old; young, 85 years old; mature, 120 years old; and old-growth, over 200 years old. Three moisture conditions were represented; wet, modal, and dry. In each stand, fungi were harvested from 25 circular 4-m^2 plots layed out on transects in a 5-ha area. All collections were identified to species, oven dried, and weighed.

Mammals were trapped in ten stands during November, concurrently with collection of fungi. For each stand, 256 snap-traps were set on a 5-ha grid. Animals were frozen until dissection. Portions of terminal fecal pellets and stomach contents were examined microscopically and fungal spores identified to genus by use of synoptic keys developed by J.M. Trappe (unpub.).

Results and Discussion

Comparison of fungal genera collected by deer mice and by mycologists shows that mice were more efficient (Table 1). Average number of genera collected per stand was 6.9 and 3.0 for mice and mycologists, respectively.

Fifteen genera and 39 species of fungi were collected. Thirty-one species occurred in fall and 14 in spring. This difference resulted largely from the numerous fall species of Rhizopogon, Leucogaster, and Tuber. Six species fruited both seasons but in each case production was disproportionate. This indicates that each of these species lack the physiological capability to fruit in equal abundance twice a year.

Although sporocarp production is generally reported as biomass (e.g., g dry wt./ha), number of sporocarps per hectare more meaningfully measures availability to mycophagists. On the basis of sporocarp number, the three species producing most abundantly in spring were: Hysterangium separabile, Leucophleps magnata, and L. spinispora. Most abundant in fall were Hysterangium cistophilum, Rhizopogon villosulus, and R. colossus. Combined data for spring and fall show that modal mature and modal young stands produce the most sporocarps per hectare.

Species number differed by habitat type and season. Maximum species diversity occurred during fall in wet, old-growth stands and only one species fruited on clearcut stands.

Conclusions

Deer mice are the most ubiquitous rodents in North America. Although they eat a wide selection of foods, including seeds of conifers, berries, and invertebrates, they also consume a variety of hypogeous fungi and thus provide a mechanism for dissemination of mycorrhizal fungus inoculum. Thousands of dollars have been spent to poison deer mice because they eat seeds of conifers. Given the ecological role of deer mice as disseminators of mycorrhizal fungi, the value of poisoning programs must be re-evaluated.

Table 1. Frequency of genera collected in ten stands.

		
Zygomycetes		
Endogone	5	2
Glomus	9	1
Ascomycetes		
Balsamia	?	0
Barssia	?	0
Elaphomyces	1	1
Genabea	1	0
Genea	1	0
Geopora	9	0
Peziza	1	0
Tuber	7	2
Basidiomycetes		
Alpova	?	0
Destuntzia	1	0
Gautieria	3	2
Hymenogaster	3	0
Hysterangium	3	3
Leucogaster	9	3
Martellia	2	2
Melanogaster	4	0
Rhizopogon	10	9
Truncocolumella	?	5

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RODENT PELLETS AS ECTOMYCORRHIZAL INOCULUM FOR TWO TUBER SPP.

By

Steven L. Miller

Keywords--Spore activation, germination

Introduction

Hypogeous fungi form sporocarps at or below the surface of the ground. Such fungi are all putatively ectomycorrhizal and in some cases comprise a major food source for many small mammals and marsupials (Trappe and Maser 1977). The intricate interrelationships among fungi, animals and trees have engendered many questions concerning the ecosystematic roles of these organisms (Fogel and Trappe 1978), yet much of the basic biology surrounding their life history remains unstudied.

The purpose of this paper is to examine the effect that passage of hypogeous fungal spores through a rodent digestive tract has on ectomycorrhizal formation. Percent mycorrhizal formation was used as an indicator of spore activation, based on spore-pellet exposure to three treatments.

Methods and Materials

Fresh ascocarps of Tuber shearii Hk. and T. canaliculatum Gilk. were surface sterilized. Exoperidia were aseptically removed with a razor. Glebal material was fed to solitary white-footed mice (Peromyscus leucopus) in freshly cleaned cages. Twelve hours after feeding, fecal pellets were placed in sterile Petri plates for treatment.

Three treatments and controls were performed on the pellets before their use as inoculum: 1) fresh untreated, 2) refrigerated in a moist environment for 3 mo, 3) air-dried for 3 mo. Control inoculum consisted of pellets from mice fed a standard lab-block diet.

Ten average sized, whole pellets from each treatment and control were planted in each of three 12 cm greenhouse pots filled with a 1:1 fine peat moss-sand rooting medium. Aseptically grown 1 mo old Pinus virginiana seedlings were planted in the pots and kept under greenhouse conditions for 3 mo. Seedlings were harvested, and ectomycorrhizal and non-mycorrhizal rootlets counted. Analysis of variance with Duncans' Multiple Range Test were used for analysis.

Results and Discussion

Table 1 shows the results of the experiment. The refrigeration treatment produced the highest number of ectomycorrhizae for both Tuber spp. The air-dried treatment resulted in significantly fewer ectomycorrhizae than the refrigeration treatment. Fresh and control treatments resulted in few or no ectomycorrhizae and were not significantly different from each other. The Tuber ectomycorrhizae were distinguished by their distinctive morphology (Figs. 1 and 2).

Passage of Tuber spp. spores through the digestive tract did not change the external appearance of the spores. Intact asci and spores were commonly observed. These data suggest that spores of Tuber spp. are not capable of germinating in the fresh condition. It appears that no direct activation of spores occurs by passage through rodent digestive tracts. Spore activation may be a function of biotrophic organisms working to deteriorate the spore wall or the result of spore after-maturity, both requiring an extended period of time before germination.

Table 1. Per cent ectomycorrhizal formation of pine seedlings inoculated with rodent pellets containing Tuber spp. spores.1/

Spore-Pellet Treatment	Fungus sp T. shearii	T. canaliculatum
Control	0a	0a
Fresh	2a	0a
Refrigerated (3 mo)	79b	61b
Air-Dried (3 mo)	34c	21c

1/Data represent means of three replicates. Letters within columns are significant differences according to Duncans' Multiple Range Test.

Figure 1. T. canaliculatum P. virginiana ectomycorrhizae; x12.

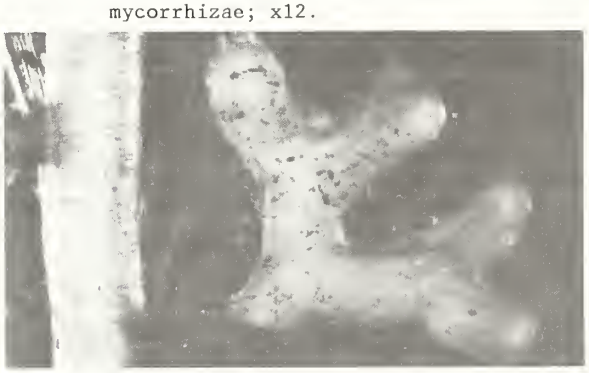

Figure 2. T. shearii P. virginiana ectomycorrhizae; x 25.

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THE ROLE OF THE FALLEN TREE IN AN OLD-GROWTH FOREST

By

Michelle T. Seidl

Keywords--Pseudotsuga menziesii, rotten wood, small mammal habitat, mycorrhizal fungi, nitrogen, decomposition.

Introduction

Large fallen trees will have to be man aged as a resource to maximize tree growth and maintain forest diversity. Complex interactions occur between fallen trees and the surrounding old-growth forest. During decomposition, fallen trees provide moisture, nutrients, and a myriad of habitats for organisms that are integral to ecological forest processes.

Discussion

In old-growth forests of the Pacific Northwest, large amounts of biomass (predominantly wood) accumulate due to large, long-lived trees, such as Douglas-fir (Pseudotsuga menziesii), western hemlock (Tsuga heterophylla), and western redcedar (Thuja plicata). Typical standing crops for old-growth Douglas-fir/western hemlock forests in the Cascade Range are 610-1084 Mg/ha with maximal values of 1449-1693 Mg/ha (Table 1). Annual productivity is also high. Slowly nutrients are released, recycled primarliy by organisms near the soil surface.

A fallen tree equates to one of five decomposition classes (Table 2). The extent of decay determines a fallen tree's function in the forest. The more decomposed trees, those in classes 3-5, are most important ecologically.

Fallen trees can be classified as structural or functional. Structural roles refer to such things as the habitats, which allow the flora and fauna to exist. Functional roles include the ways in which energy and nutrients are cycled within the system.

As a fallen tree decomposes, nitrogen concentration (percent dry weight) increases. Class 2 fallen trees have an average nitrogen concentration of 0.10, compared to 0.25 for class 5. Mycorrhizal fungi aid nitrogen accumulation by acting as nutrient sinks.

Wood density decreases as decay continues. Class 1 fallen trees have an average density of 500 mg/cm^3, whereas that of class 5 is 100 mg/cm^3. Average water concentration (percent dry weight) increases sharply from class 1 (100%) to class 4 (220%). This is followed by a slight decrease in class 5 (180%). High moisture content of fallen trees makes them particularly important as amphibian habitat.

Forests are constantly changing in structure and composition. The fallen tree is a major structural component in the old-growth forest and is important ecologically to the aquatic as well as the terrestrial environment. Large, fallen trees in streams control habitat distribution, stability of streambeds and streambanks, and routing of sediments and water through the system (Franklin et al. 1981). By providing needed habitats and controlling the rates at which energy, nutrients, and water flow through the ecosystem, the fallen tree becomes a source of structural and functional diversity.

Table 1. Biomass average value comparisons for different forest types. (Adapted from Fogel and Hunt 1979 and Franklin 1982).

Forest type	Biomass (Mg/ha)
Old-growth Douglas-fir (200+ years)	1151
Young Douglas-fir (30-50 years)	89
Temperate deciduous (hardwood)	295
Tropical rain forest	385

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Table 2. Decay classification of Douglas-fir (Pseudotsuga menziesii) logs. (Adapted from Maser and Trappe 1984).

Log characteristics	Class 1	2	3	4	5
Bark	intact	intact	trace	absent	absent
Twigs 3 cm (1.2 in)	present	absent	absent	absent	absent
Color of wood	original color	original color	original to red brown	reddish or light brown	red-brown to dark brown
Mycorrhizae	none	none	none	in sapwood	in sapwood and heartwood

MYCOPHAGOUS AMOEBAE REDUCTION OF ROOT COLONIZATION BY RHIZOPOGON

By

S. Chakraborty, C. Theodorou and G.D. Bowen, CSIRO, Adelaide, Australia.

Keywords--Pinus radiata, Saccamoeba sp., Gephramoeba sp., rhizosphere

Introduction

Although mycophagous amoebae are frequent colonizers of the rhizosphere, there has been little experimental study of their impact on the rhizosphere population. However they have been shown to reduce the severity of take-all disease of wheat[1]. This study examined the effect of 3 mycophagous amoebae on rhizosphere colonization by the ectomycorrhizal fungus, Rhizopogon luteolus.

Methods and Materials

Sterile Pinus radiata seedlings were grown in sterile soil in plugged tubes and inoculated with washed mycelia (on agar) of R.luteolus[2]. Three species of amoebae isolated from soil by S. Chakraborty and grown in the presence of Klebsiella sp. were suspended in saline and inoculated to the root: a. at the time of mycorrhiza inoculation, b. two weeks after mycorrhiza inoculation or c. mixed in soil in different concentrations, at the time of mycorrhizal inoculation. Plants were grown for 4 weeks at 24°/16°C, 12 hr day. Mycorrhizal fungi were then stained and the length of root colonized was measured[2]. There were 30 replicate plants per treatment.

Results

(1) The lengths of root colonized at 4 weeks by R.luteolus (R.l.)when amoebae were added at the same time as R.luteolus and two weeks later are given below.

Treatment	Length colonized (mm)	Colonization rate mm day^{-1}
a. Amoebae and R.l. inoculated together		
R.l. (+ Klebsiella)	18.4^{a}*	0.58
+ Saccamoeba sp.	5.3^{b}	0.17
+ Gephramoeba sp.	8.4^{b}	0.26
+ unidentified Leptomyxid	17.8^{a}	0.56
b. Amoebae inoculated 2 weeks after R.l.		
0-2 weeks - R.l. alone		0.97(0-2 weeks)
2-4 weeks, R.l., + K + mixture of amoebae		0.18(2-4 weeks)

* a is significantly greater than b at P=0.001

(2) The length of root colonized by R.luteolus when the soil was inoculated with Saccamoeba sp. at populations commonly occurring in soil are given below:

Amoebae added g^{-1} soil	Length root colonized (mm)
0	12.9^{a}*
42.3	12.9^{a}
84.6	11.3^{a}
423	8.0^{b}
846	5.8^{b}

* a is significantly greater than b at P=0.01

The R/S ratio of amoebae was 20:1 indicating the amoeba was an active coloniser of the rhizosphere.

Conclusions

1. Some mycophagous ameobae in the rhizosphere can markedly depress establishment of ectomycorrhizal fungi.

2. Protozoa have potential importance in determining the microbial composition of the rhizosphere. There is a need for more similar studies in soils.

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EFFECTS AND INTERACTIONS OF SLASH BURNING AND MYCORRHIZAL INFECTION ON DOUGLAS-FIR SEEDLING GROWTH AND MORPHOLOGY

By

C.H. Black

Keywords- Pseudotsuga menziesii, nitrogen, phosphorus, carbon allocation, shoot:root ratio.

Introduction

Mycorrhizae can aid in plant uptake of P and other insoluble mineral nutrients. Carbon and mineral nutrient requirements of mycorrhizal fungi may shift cost:benefit ratios of infection under differing soil nutrient conditions. This study quantified mycorrhizal effects on the growth response and C allocation patterns of Douglas-fir seedlings growing in soils with different nutrient availabilities as a consequence of mineralization by slash burning.

Materials and Methods

Douglas-fir seedlings were grown in pots for 24 weeks in soils collected from unburned, mild burn, and hard burn areas of an Oregon Coast Range clearcut. On harvest mycorrhizal infection was quantified for 77 seedlings, and plants were separated into component parts, dried, weighed, and analyzed for mineral nutrient content.

Results and Discussion

Soil tests showed that available soil nutrients increased with increasing burn intensity. Partial correlation of foliar nutrient concentrations versus seedling weight suggested that both N and P were limiting in unburned and mild burn soils, but only P was limiting in the hard burn soil.

Percent mycorrhizal infection increased with burn intensity (Figure 1). Growth also showed a positive correlation with burn intensity (R^2 = .581, p .001). Within burn catagories, growth showed a positive correlation with infection only in the hard burn soil.

Percent mycorrhizal infection had a strong effect on carbon allocation patterns in each soil, reducing shoot:root ratios in the unburned and mild burn soils, and increasing them in the hard burn soil (Figure 2).

Percent infection strongly affected the morphology of seedlings in the hard burn soil (Figure 3):
% Infection = ln(leaf wt/total wt) + (stem wt/tot wt) + (root wt/tot wt) + (short root wt/tot wt) + ln(myc short root wt/tot wt) R^2 = .864, p .001.

Mycorrhizal infection appeared to benefit plants under P limiting, but not N or N and P limiting conditions. Percent infection was restricted where infection was disadvantageous in terms of reducing rather than increasing shoot:root ratios.

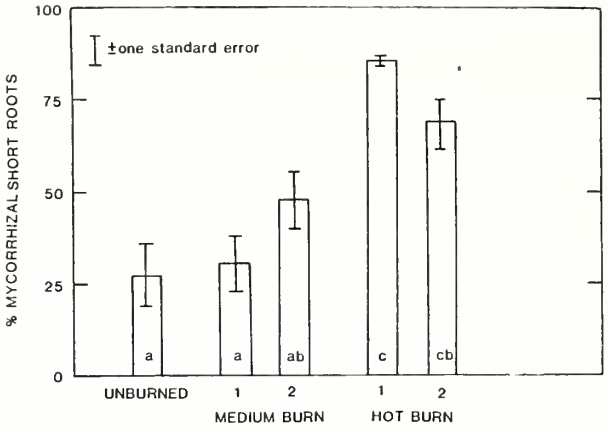

Figure 1. Percent mycorrhizal infection vs burn intensity on NW (1) and SE (2) aspect collected soils from the clearcut.

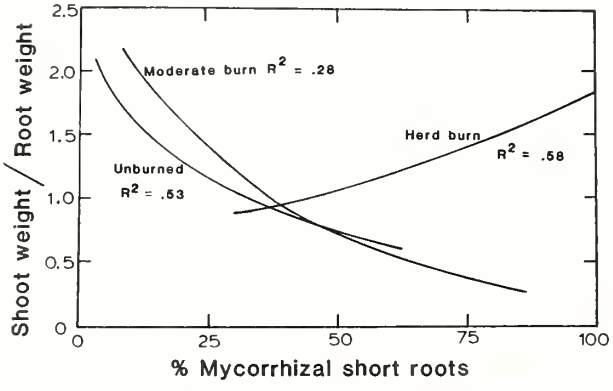

Figure 2. Shoot:root ratios of seedlings versus percent mycorrhizal infection over the ranges of infection observed in the three soil catagories.

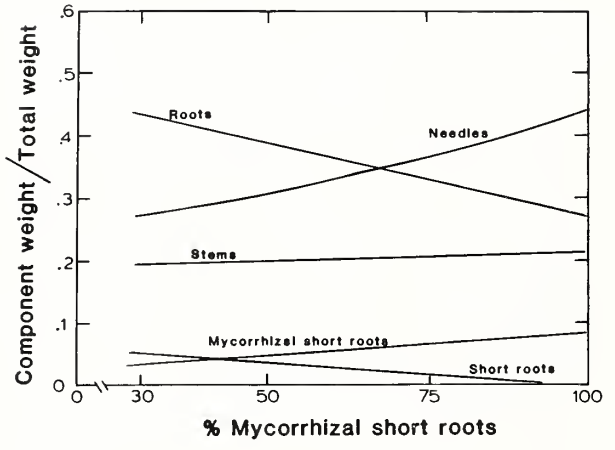

Figure 3. Model of seedling morphology versus percent infection for the seedlings in the hard burn soil.

DYNAMIC BALANCE IN PINE FORESTS--GROUND COVER--MYCORRHIZAE-TREES

By

R. T. Brown

Keywords--Cladina, Pine reproduction, Allelopathy
Mycorrhizae, Tree growth

Introduction

Pine trees grow slowly, if at all, where a reindeer lichen mat exists when seeds fall. (Fig. 1 and 2).

Methods and Materials

Petri dish culture of mycorrhizal fungi with and without reindeer lichen extract, and nursery growth plots of scotch pine and Norway spruce with and without reindeer lichen cover were established. Field observations both initiated the investigation and confirmed the results.

Results and Discussion

Petri dish culture showed that all reindeer lichen species tested inhibited growth of many but not all mycorrhizal fungi. Cladina stellaris is the most inhibitory lichen. Figure 3 illustrates the lack of myocrrhizae with C. stellaris cover.

Figure 1. Foreground, reindeer lichen cover. Background, moss with trees.

Figure 2. 13 year old jack pine without reindeer lichens (left), with lichens (right); a 20-fold difference in tree size.

Reindeer lichens grow best in partial shade. Inhibition of mycorrhizae by lichen chemicals controls tree growth and benefits lichens. Reindeer eat lichens, reducing inhibitor production, thus increasing tree growth. Humans influence reindeer and predator numbers, harvest timber, and change fire frequency. Knowledge of lichen-mycorrhizae-tree relationships makes better forest management possible.

Table 1. Tree densities with lichen and moss ground cover shown in Fig. 1. A 9 fold difference.

Cover type	Number of trees	Area occupied	Area per tree
Lichen	19	611m^2	35.2m^2
Moss	392	1389	3.5

Figure 3. Nursery culture of Norway spruce with (right), and without (left) Cladina stellaris cover.

Figure 4. Foreground, Scotch pine seedlings, age 21 years, 27 mycorrhizal tips. Road edge seedlings, age 6 years, 195 mycorrhizal tips/seedling.

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ENDEMIC ECTOMYCORRHIZAL FUNGI OF PONDEROSA PINE IN THE CENTRAL PLAINS

By

M. Melichar, B. Daniels Hetrick, and W. Geyer

Keywords--Pinus ponderosa, Suillus, Rhizopogon, pure culture synthesis, Great Plains

Introduction

Ponderosa pine (Pinus ponderosa Laws.) is planted throughout the Great Plains in nursery research, windbreak, shelterbelt, and landscape plantings. Many of these sites are prairie or grassland soils on which trees have never existed. Consequently, they are deficient in ectomycorrhizal forming fungi. Riffle and Tinus (1982) suggest that endemic ectomycorrhizal fungi may be more efficient in Great Plains soils than non-native species. However, pure culture of endemic ectomycorrhizal fungi for use in the Great Plains have not been developed because the fungi have not been identified. The objectives of this research are to:

(1) Collect and identify the mycorrhizal fungi from ectomycorrhizae and sporocarps of ponderosa pine from seven locations in the Central Great Plains.
(2) Determine capability of suspect ectomycorrhizal fungi to synthesize ectomycorrhizae on ponderosa pine seedlings in pure culture.
(3) Determine environmental parameters for growth of confirmed ectomycorrhizal symbionts on culture media.

Methods and Materials

Hypogeous and epigeous sporocarps and ectomycorrhizal rootlets of ponderosa pine were collected from six Nebraska and one Kansas ponderosa pine planting and nursery sites (Figure 1). Isolations were made from each sporocarp/ecto-rootlet onto Hagems agar medium amended with antibiotics and suspect cultures were tested in pure culture conditions. An aseptically germinated ponderosa pine seedling and a fungus were introduced into a 300 x 38 mm glass culture tube containing 100 ml of vermiculite, 10 ml of peat moss, and 60 ml of Hagems nutrient solution. Seedlings were subjected to a 16 hour photoperiod (20° C daytime, 18° C nights) with 1600 - 2000 foot candles light. After approximately six months rootlets of each seedling were placed on culture media and subsequent cultural growth characteristics compared to those of the initial inoculum to verify that no contamination had occurred. Other rootlets were freeze-sectioned, stained in cotton blue in lactophenol, and examined microscopically to confirm the presence of fungal mantle and Hartig-net.

Results and Discussion

Five fungal isolates have been confirmed in pure culture trials (Table 1). The fact that Rhizopogon ponderosa, Suillus sp., Tuber asa, and Cortinarius sp. have each been collected from several sites suggests that they may be adaptable to climatological and soil conditions found in the Great Plains. Such ecological adaptability is important for ectomycorrhizal inoculation of ponderosa pine and targeting of fungal inoculum for use across the highly variable environmental conditions encountered in the Great Plains.

Further tests will be conducted on all confirmed fungal isolates to determine (1) temperature range and optimum temperature, (2) optimum pH and pH tolerance, and (3) tolerance to a range of water potentials of each confirmed ectomycorrhizal fungi. Additionally, controlled environment/greenhouse and outplanting studies will be conducted to determine ecological adaptation and outplanting performance of confirmed ectomycorrhizal fungi in the various climatic and soil conditions of the Great Plains.

Figure 1. Nebraska and Kansas ponderosa pine planting and nursery sites from which hypogeous and epigeous sporocarps and ectomycorrhizal rootlets were collected.

Table 1. Sporocarps/rootlets collected and established in pure culture.

Fungal Species (Tentative identification)	Collection Location	Pure Culture Trials Confirmed	Pending
1982 Collection			
Rhizopogon ponderosa	Plattsmouth	X	
Rhizopogon ponderosa	Junction City	X	
Rhizopogon ponderosa	Hastings	X	
Suillus granulatus	Chadron	X	
Suillus granulatus	Junction City		X*
Suillus fuscotomentosus	Junction City		X*
Tuber asa	Plattsmouth	X	
Tuber asa	Hastings		X*
Phallus ravenelii	Halsey Forest		X
Helvella leucomeleana	Plattsmouth		X
Leucopaxillus gentianeus	Chadron		X
Amanita vaginata	Chadron		X*
1983 Collection			
Cortinarius sp.	Chadron		X*
Cortinarius sp.	Hastings		X*
Cortinarius sp.	Plattsmouth		X*
Lycoperdon foetidum	Hastings		X
Lycoperdon perlatum	Chadron		X
Lycoperdon perlatum	Plattsmouth		X
Calvatia cyathiformis	Hastings		X
Bovista pila	Hastings		X
Leucoagaricus naucina	Hastings		X
Hygrophorus pratensis	Chadron		X*
Collybia sp.	Plattsmouth		X
Lepista tarda	Plattsmouth		X

*Literature suggests that these fungi are from families known to form ectomycorrhizae.

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ENGLISH OAK ECTOMYCORRHIZAE AND ROOT DISTRIBUTION

By

R. V. Gessner and H. Zare-maivan

Keywords--Quercus robur, ectomycorrhizae, root weight, percent mycorrhizal infection, linear regression

Introduction

In the USA, English oaks are planted primarily as ornamental trees and in forest plantations (Johnson 1981). Little quantitative information is available on the ectomycorrhizae and root systems of mature trees, and any relationships that may exist between the ectomycorrhizae and roots of these trees. The purpose of this study was to determine by using linear regression analysis any significant correlations that occur in the distribution of the ectomycorrhizae and roots of two intensely sampled English oaks.

Methods and Materials

Two English oak trees growing in meadow at the Morton Arboretum, Lisle, Illinois were sampled during June 1982. A cylindrical steel corer was used to take 10 cm X 10 cm soil samples under the canopy. Sixteen equidistant core samples were taken along each of two concentric circles with radii extending from the center of the tree trunk to the edge of the canopy (C) and half the distance to the edge of the canopy (C/2). Soil particles were washed from the roots and any non-oak roots were removed. Mycorrhizal tips and total root tips were counted from each sample. Percent mycorrhizal infection, total root dry weight, and fine root dry weight were also determined.

Results and Discussion

Significant correlations were found between a number of parameters measured (Table 1,2). In tree H2, the number of mycorrhizal tips was correlated with the dry weight of the fine roots at both C and C/2. For tree D2, there was a significant correlation with the total root dry weight at C and C/2 but not with fine root weight at C/2. The number of root tips in tree D2 was correlated with total root dry weight and fine root dry weight at C and C/2. In tree H2 only the fine root weight at C and C/2 was significantly correlated with the number of root tips. The number of mycorrhizal tips was significantly related to the total number of root tips in both trees. The percent mycorrhizal infection for both trees was not significantly correlated with any of the other parameters measured.

From the results of this study it can be concluded that there is a relationship between the number of mycorrhizal/root tips and root biomass. This relationship, however, appears to be different between trees and between locations under the canopy since linear regression lines were not the same. Percent mycorrhizal infection as has been found by others (Grand and Harvey, 1982) for tree seedings did not correlate with growth factors while a correlation has been demonstrated between the number of mycorrhizal tips and root biomass.

Table 1. Mean, correlation coefficient (r), slope (m) and y-intercept for ectomycorrhizal and root parameters for tree H2. All correlation coefficients are significant at $p \leq 0.05$ based on the t-test.

Parameter	$\bar{x}$	r	m	b
Edge of canopy				
Mycorrhizal tips vs	110			
Fine root dry wt (g)	0.10	0.68	1,056.4	-1.9
Root tips vs	210			
Mycorrhizal tips	110	0.97	1.8	21.1
Fine root dry wt (g)	0.10	0.72	2,044.8	-1.4
Half canopy				
Mycorrhizal tips vs	113			
Fine root dry wt (g)	0.13	0.60	613.1	23.0
Root tips vs	237			
Mycorrhizal tips	113	0.87	1.9	34.1
Fine root dry wt (g)	0.13	0.62	1,332.2	47.6

Table 2. Mean, correlation coefficient (r), slope (m) and y-intercept for ectomycorrhizal and root parameters for tree D2. All correlation coefficients are significant at $p \leq 0.05$ based on the t-test.

Parameter	$\bar{x}$	r	m	b
Edge of canopy				
Mycorrhizal tips vs	146			
Tot. root dry wt (g)	0.50	0.58	97.7	90.7
Root tips vs	277			
Mycorrhizal tips	146	0.89	2.0	5.7
Tot. root dry wt (g)	0.50	0.72	275.6	139.1
Fine root dry wt (g)	0.18	0.63	894.4	117.0
Half canopy				
Mycorrhizal tips vs	528			
Tot. root dry wt (g)	0.72	0.66	474.3	184.8
Fine root dry wt (g)	0.50	0.80	882.2	77.9
Root tips vs	832			
Mycorrhizal tips	528	0.97	142.0	81.0
Tot. root dry wt (g)	0.72	0.67	692.5	330.3
Fine root dry wt (g)	0.50	0.82	1327.6	154.0

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EFFECT OF SOIL WATER POTENTIALS ON ENDO- AND ECTOMYCORRHIZAE IN POPULUS DELTOIDES

By

D. J. Lodge

Keywords--Eastern cottonwood, Salix nigra x ?, Glomus fasiculatus, Gigaspora calospora, G. margarita, flooding, competition

Introduction

A few plant species are known to regularly form mycorrhizae with both VA-endo- and ectomycorrhizal fungi, including Populus deltoides Bartr.(eastern cottonwood). Such plants are ideal for determining whether endo- and ectomycorrhizal infection are favored by different environmental conditions.

Methods and Materials

Eastern cottonwood cuttings (Stoneville 261 clone) were planted among endo- plus ectomycorrhizal willow sprouts and grown for six weeks. The cottonwood cuttings were then pruned to one leader and transplanted to four-inch diameter PCV pipes containing seived, well mixed Chewacla fine sand silt loam. Every third fine root was preserved for determination of initial infection levels. The pipes were of four heights (15, 30, 40, and 60 cm), with six replications of each height. Trees which died during the first two weeks were replaced. The transplanted cuttings were initially watered once from the top, but subsequently received water only through holes at the base from a 10 cm-deep pool of water; water potential gradients were thereby maintained in each tube throughout the experiment. A plastic rainfly was sealed around each stem to prevent water from entering at the top, and the trees were grown next to a greenhouse (south wall) for three months. At harvest, the intact soil collums were forced from the tubes and divided into 15-cm sections for sampling (Levels I to IV, wet to dry). Percentage of soil moisture was measured for each section, and was also determined for the soil used at -0.1, -0.5, -1, -5, -15, and -30 bars. In addition, five eastern cottonwood and six hybrid willow trees (Salix nigra x ?) were sampled along drainage gradients in Raleigh, N.C. three days after heavy rains, and soil water potentials were measured using a thermocouple psychrometer.

Samples were stained in lactophenol with analine blue and percentages of endo- and ectomycorrhizal root lengths were estimated using the grid intersect technique. Dual mycorrhizae were included in estimates of both endo- and ectomycorrhizal root length, and terminal and non-terminal roots $<$ 2mm diameter were tallied separately. At least 115 cm of root length was examined, and at least 200 observations were made for each sample at x160 and x1,000.

Results and Discussion

Ectomycorrhizae. An analysis of varience (ANOVA) was used to determine the effects of level (I-IV), position (top vs not top), and level by position interaction on the percentage of terminal root length infected by ectomycorrhizal fungi (TIPECT). Percentages were normalized using the arc sine transformation. The ANOVA model was statistically significant at p=0.0001 (r^2=0.66); level and position (but not their interaction) contributed significantly to the model (Table 1). Ectomycorrhizal infection was very low in the water logged section (Level I), high in the moist section (Level II, 0 to -0.1 bars), and almost totally absent from the drier sections (Levels III and IV, -0.1 to -8.1, and -7.7 to -34 bars, respectively). Paired t-tests were used to compare levels within tubes; levels I vs II, and II vs III were statistically different from one another at p=0.001, levels I vs III were different at p=0.025, and levels III vs IV were not different. These data suggest that for the natural inoculum used in this study, colonization of P. deltoides roots was greatest under very moist, but not water logged conditions. Top sections had fewer ectomycorrhizae than comparable non-top sections; there may have been unfavorably high temperatures in top sections due to greater solar irradiation. An analysis of covarience indicated that initial infection level, position, and moisture level (both linear and quadratic terms), all contributed significantly to the model ($p > F$ ea. < 0.05; overall $p > F$=0.006).

Table 1. ANOVA table for ectomycorrhizal infection versus level, position (top or not top), and level x position interaction. Water potentials graded from 0 bars in level I to -7.7 - -34 bars in level IV.

Source	DF	S.SQ	Mean Sq	F	P > F
Model	6	1.281	0.213	14.62	0.0001
Error	46	0.672	0.015		
Level	3	1.100		25.12	0.0001
Position	1	0.217		14.84	0.0004
Interaction	2	0.000		0.000	1.0000

Endomyorrhizae. ANOVA proceedures were used to determine the effect of tube level on the arc sine transformed percentage of the total root length infected by VA-endomycorrhizal fungi (TOTEND); level was highly significant in the analysis (p= 0.0001, r^2=0.50). Position (top vs not top) did not significantly influence endomycorrhizal infection, according to t-tests within levels. TOTEND was lowest in the water logged (I) and driest (IV) levels, which did not statistically differ from one another (t-test, paired within tubes). TOTEND was moderate in level II and highest in level III. Infection in levels I vs II, and II vs III were statistically different at p=0.001, and levels III vs IV were different at p=0.02. Both the linear and quadratic terms were significant in a multiple linear regression of TOTEND versus percent moisture ($p > T$ =0.0002, each). Endomycorrhizal infection was least at the extreams of the moisture gradient, but was still much higher than ectomycorrhizal infection in all except level II. Identification of spores and accessory vesicles suggest that Gigaspora calospora, G. margarita, and Glomus fasciculatum were the predominant endomycorrhizal fungi.

Transects. Results from the native willow and cottonwood transects were similar to those from the controlled experiment, except that endomycorrhizal infection was reduced where ectomycorrhizal infection was high. This may suggest competitive interactions among the fungi.

VAM SPORE ABUNDANCE AND DIVERSITY IN AN ILLINOIS CORN FIELD AND ADJACENT TALLGRASS PRAIRIE

By

Roger C. Anderson and Anthony E. Liberta

Keywords--*Glomus*, *Gigaspora*

Introduction

Occurrence and importance of vesicular-arbuscular mycorrhizae (VAM) in agronomic systems have been documented by numerous workers. However, few workers have compared mycorrhizal associations in native ecosystems and the agricultural systems that replaced them (Hetrick and Bloom, 1983). This study compares VAM fungal spore abundance and diversity in a 2.1 ha remnant native tallgrass prairie and an adjacent corn field.

Methods and Materials

Prairie vegetation and VAM spores were sampled between July 16-21, 1982. Ten 25 cm X 25 cm quadrats were located at approximately m intervals along each of 5 line transects using random sampling procedures. Within each quadrat, aerial cover of vascular plants rooted in the quadrat was estimated and 3 soil cores 2 cm in diam and 25 cm deep were randomly collected. Soil cores from each quadrat were air-dried and thoroughly mixed. Soil from each quadrat (20 g) were combined by transect and analysed for texture, pH, available Ca, Mg, K and P, and total N and organic matter. Each quadrat also provided 20.8 cc soil for spore counts and identification. Only intact, cytoplasm-filled spores were counted, but all spores were identified. Tests for association between the occurrence of VAM species and prairie plants within the 50 quadrats were performed (Hurlbert, 1969). Spore count data for *G. fasciculatum* were not included because spores of this species occurred in every quadrat. Within the corn field, 10 samples (each comprised of 3 cores) were taken on July 21 at approximately 25 m intervals along a line 10 m from the edge of the prairie that followed its west and south sides.

Results

The dominant plant species on the prairie site is prairie dropseed (*Sporobolus heterolepsis*) but the site has a diverse mixture of forbs that include rattlesnake master (*Eryngium yuccifolium*), sunflower (*Helianthus hirsutus*), wild quinine (*Parthenium integrifolium*) and smooth aster (*Aster laevis*). Spores of 6 VAM species were isolated from prairie soil and 4 from the corn field. Significant differences in mean spore counts occurred between prairie sites and between some prairie sites (2,4) and the corn field (Table 1).

There were 5 significant associations found between prairie plants and the VAM spores, but this is slightly fewer than expected by chance alone at the 0.05 probability level.

Corn field soil had slightly more sand (17% *vs* 1-7%) and less silt (54% *vs* 66-76%) than prairie soil. Clay content and levels of K and Mg were similar for prairie sites and the corn field. Corn field soil had higher pH, more available P and Ca, and less organic matter and total N than prairie sites. Available Ca and spore abundance were negatively correlated ($r_s = -0.94$, $p < 0.05$, $n = 6$).

Discussion

Conversion of prairie to corn field had little influence on VAM abundance or diversity. Of 6 VAM species isolated from prairie soil, only 2 did not occur in the corn field (*G. mosseae* and *G. calidonium*. Hetrick and Bloom (1983) reported a greater diversity of species in prairie soil than in cultivated wheat soil. We observed similar VAM diversity in the corn field *vs* prairie that may be partially due to the ability of corn to form VAM with many species of Endogonaceae.

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Table 1. Mean spore counts for the five prairie samples and the corn field

VAM Species	Prairie Transect Number 1	2	3	4	5	Corn Field
Glomus fasciculatum	38.6 ± 34.9	84.8 ± 32.4	53.0 ± 34.8	91.6 ± 51.3	34.3 ± 54.2	26.0 ± 15.5
G. geosporum	0.7 ± 0.8	1.8 ± 1.3	0.9 ± 1.2	2.9 ± 5.2	0.8 ± 1.3	0.1 ± 0.3
Gigaspora calospora	1.2 ± 1.5	0.3 ± 0.5	1.2 ± 0.9	1.0 ± 1.2	0.6 ± 0.7	0.5 ± 0.8
Glomus mosseae	0	0.1 ± 0.3	0	0.4 ± 0.7	0.2 ± 0.6	0
G. caledonium	0	0.1 ± 0.3	0	0.2 ± 0.4	0	0
Gigaspora margarita	0.1 ± 0.3	0	0	0	0	0.1 ± 0.3
All species ($\bar{X}$ ± S)*	40.6 ± 36.2^{c}	87.3 ± 31.5^{ab}	55.1 ± 34.5^{bc}	96.1 ± 49.3^{a}	35.9 ± 53.9^{c}	27.7 ± 15.9^{c}
Number of species	4	5	3	5	4	4

*Oneway ANOVA indicated that there were significant differences ($p < 0.001$) between samples. Sample means with the same letter do not differ significantly at $p < 0.05$ (Duncan's multiple range test).

MYCORRHIZAL FUNGI ASSOCIATED WITH SEA OATS IN FLORIDA SAND DUNES

By

David M. Sylvia

Keywords--Acaulospora, Gigaspora, Glomus, Uniola paniculata, distribution, revegetation, sampling

Introduction

Sea oats (Uniola paniculata L.) are the dominant dune-stabilizing plants in the southeastern U.S. and have been used widely in coastal revegetation. However, transplant survival and growth are reduced by wind, salt spray, excessive sand erosion or accretion, and extremes in temperature, moisture and nutrient levels. To overcome environmental stresses and obtain rapid growth, these pioneer dune plants may depend on beneficial root-associated microorganisms such as vesicular-arbuscular mycorrhizal fungi (VAMF).

The VAMF have been collected from sand dunes along Lake Huron, the Rhode Island shore, Australia, Scotland and Italy. As part of a study to understand the role of VAMF in the establishment of sea oats in Florida, the distribution of these fungi in pioneer dunes was determined.

Methods and Materials

Samples were collected in November, 1983 from Fort Clinch State Park and Anastasia State Recreation Area in northeastern Florida. Three plots (2 x 1 m, oriented parallel to the shore) were established at each site on pioneer foredunes that were colonized by sea oats. Thirty cores (2.5 x 40 cm) were taken from each plot along diagonal transects. There were 6 transects per plot and 5 samples per transect. The top and bottom halves of each sample were placed in separate plastic bags and transported to the laboratory for analysis.

Spores of VAMF and roots were separated from the sand by wet-sieving and decanting. Spores were collected on sieves that had 45 and 90µm openings. Roots were cleared in hot, 10% KOH and stained with 0.05% typan blue. Root length and percentage of root length colonized by VAMF were estimated by the gridline-intersect method. Spore counts for each VAMF species, total spore counts, and the cm of colonized roots per 100 g of sand per sample were used to construct frequency tables. Frequency data were tested for goodness-of-fit to several discrete frequency distributions using a computer program developed by Gates and Ethridge (1972).

Results and Discussion

Seven species of VAMF were collected from pioneer sand dunes colonized by sea oats in northeastern Florida. The most abundant species was Glomus fasciculatum (deserticola type) followed by Acaulospora scrobiculata and Gigaspora weresubeae.

The total spore density ranged from 0 to 557 per 100 g per sample. The variance exceeded the mean in all observed frequency distributions (for each VAMF species independently and for total spore counts). The observed frequencies of total spores per sample were best described by the negative binomial distribution, with k values ranging from 0.042 to 0.975 . The k parameter is an index of dispersion, where a small value (<2.0) indicates a contagious or aggregated population (Southwood, 1978).

The distribution of colonized roots in the dunes was also nonrandom. The data were best described by the negative binomial and Neyman type A (Skellam, 1958) distributions, indicating extreme aggregation.

Contagious distributions are common in ecological sampling (Southwood, 1978) and have been used to describe populations of soilborne plant pathogens (Martin et al., 1983; Hau et al., 1982). Spores of VAMF and colonized root pieces are likely aggregated, and sampling methods that assume a random distribution may be in error. The effects of aggregated population distributions on sampling technique and statistical analyses are discussed by Southwood (1978) and Hau et al. (1982).

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EFFECT OF RHIZOBIUM STRAIN ON RESPONSE OF BERSEEM CLOVER TO MYCORRHIZAL FUNGI

By

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Keywords--nitrogen, phosphorus, salinity

Introduction

Many forage legumes growing on low input sites are exposed to both N and P deficient soils, making it essential that both mycorrhizal and rhizobial symbioses are effective.

In addition, in many irrigated locations especially there is potential for accumulation of excess soluble salts. Thus plants are confronted with additional stress due to low solution osmotic potentials.

The following experiment was designed to test the response of berseem clover (Trifolium alexandrinum L.) to the presence of mycorrhizal fungi with two Rhizobium strains under moderate salinity. One strain, R. trifolii TRB4, was collected from a salt-affected soil in North Africa, and we hypothesized that N_2-fixation by plants inoculated with this organism would be less sensitive to salinity than those inoculated with a conventional isolate, 170A4.

Materials and Methods

Berseem plants were grown in modified Leonard jars, supplied with nutrient solution through a wick which passed from a bottom reservoir into the masonry sand soil. The experimental design was a complete factorial, with two mycorrhizal treatments (+ or - Glomus deserticola), three Rhizobium treatments (R. trifolii TRB4, R. trifolii 170A4, Nitragin, Inc., and no Rhizobium), and three salinity levels (1.4, 2.5, or 3.6 dS/m). Seven replicates of each treatment were arranged in a completely randomized block design and grown in a growth chamber for 10 weeks. Plants were then harvested, and fresh and dry weights of tops and roots were measured. Plant tissue was analyzed for total Kjeldahl N.

Results and Discussion

Uninoculated plants grew very poorly and are not further considered here. No contaminant infection of uninoculated plants by either mycorrhizal fungi or Rhizobium was observed.

Plants inoculated with strain TRB4 were larger in every case than those inoculated with 170A4 (Table 1). Mycorrhizal infection percentages were low in all caes, but were consistently higher in TRB4 than in 170A4. Plant P concentrations were changed little (usually slight decrease) with VAM. Nitrogen concentrations tended to be slightly lower in +VAM plants than in -VAM plants. Total N accumulation was similar for all treatments except +TRB4, +VAM, which was always highest. The latter result suggests a strong interaction between Rhizobium strain and VAM. Although the specific mechanism of this interaction is not clear from this experiment, it may be related to the energy relations of the symbionts and the plant (Paul and Kucey, 1981; Drevon, 1982).

Table 1. Growth, N, and P responses of Trifolium alexandrinum to inoculation with Rhizobium and Glomus deserticola.

Salt	Rhiz.	VAM	Dry wt	[P]	[N]	Total N accum.
			mg	mmoles	Moles	mg
dS/m			plant	kg	kg	jar
1.4	TRB4	+	584	34.9	1.69	13.85
		0	408	36.2	1.83	10.40
	170A4	+	439	37.8	1.91	11.55
		0	396	44.0	1.96	10.64
2.5	TRB4	+	721	30.9	1.44	16.00
		0	341	31.5	1.65	7.84
	170A4	+	412	33.6	1.67	9.94
		0	400	49.8	1.75	9.70
3.6	TRB4	+	536	30.1	1.56	11.70
		0	388	29.4	1.58	8.53
	170A4	+	310	35.4	1.66	7.21
		0	365	32.1	1.71	9.00

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STATISTICAL TREATMENT OF ENDOGONACEOUS SPORE COUNTS

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Keywords--*Glomus, Gigaspora, Acaulospora,* aggregation, negative binomial, logarithmic series, nonparametric statistics

Introduction

Endogonaceous spores are aggregated spatially, a fundamental and important fact that defines the optimum statistical procedures for analysis of spore counts. Because of their aggregation, spore counts have very high variances and non-normal frequency distributions (Anderson et al. 1983, Walker et al. 1982).

We used seven sets of spore counts to demonstrate the non-normality of the data, and their fit to the negative binomial or logarithmic series distributions. We then used several alternative procedures to test for differences between spore counts in two treatments of an experiment.

Methods

Collections of *Gigaspora gigaspora, G. gigantea, G. verrucosa, G. fulgida,* and *Acaulospora scrobiculata* were made on Block Island Sound, RI, and Assaleague Island, MD and VA. A collection of a *Glomus* sp. (*constricum*?) was made near Fort Collins, CO. Soil samples were taken with small soil core sampling tubes. Between 27 and 50 cores were taken for each of the collections.

Comparisons of spore counts in two treatments of an experiment (St.John et al. 1983) were made by several alternative procedures: Student's t test, Student's t test on log (x+1)-transformed data, and the Kruskal-Wallis one-way analysis of variance by ranks.

Results and Discussion

All seven spore collections had non-normal frequency distributions, as shown by tests of skewness (Snedecor and Cochran 1969). They could be shown to fit either the negative binomial distribution or Fisher's logarithmic series distribution (Bliss and Fisher 1953).

The non-parametric procedure had greater sensitivity than two variations on the parametric test (Table 1). Its results were also more reliable because of its lower sensitivity to non-homogeneity of variance (Kesselman et al. 1977).

Transformation of the data can sometimes bring spore counts into satisfactory conformity with the assumptions of parametric statistical procedures. Specialized transformations based on Taylor's power law or Anscombe's transformation for the negative binomial distribution may be optimal (Southwood 1966), but when counts resemble a logarithmic series distribution the simpler log (x+1) transformation is often adequate.

When it is not necessary to characterize the spatial distribution of spores in the field, collecting procedures can be optimized to bring the raw data into near conformity with the assumptions of parametric tests. The available methods include multiple composite samples from each treatment or plot.

Table 1. Analysis of spore counts in OM-amended and control treatments.

	Test statistic	Significance
Student t test, untransformed data	t=2.29	p<0.025
Student t test, log(x+1) transformation	t=2.39	p<0.020
Kruskal-Wallis one-way analysis of variance by ranks	H'=251	p<0.001

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INFLUENCE OF SUBSTRATE ON THE GLOMUS-FUSARIUM INTERACTION ON TOMATOES.

By

M. Caron, J.A. Fortin, and C. Richard.

Keywords--Fusarium rot , Fusarium oxysporum f.sp. radicis-lycopersici, Lycopersicon esculentum, Foot and root rot.

Introduction

Previous interaction studies between vesicular-arbuscular mycorrhizae (VAM) and fungal plant pathogens have been conducted in very different soils. Experimental conditions were thus difficult to reproduce and have often resulted in conflicting reports for the same VAM-host plant-fungal pathogen association (Dehne and Schönbeck 1975, McGraw and Schenck 1981). The objective of this study is to demonstrate the variability of the VAM-fungal plant pathogen interaction in relation with the substrate by inoculating a Glomus and a Fusarium on tomatoes in various substrates.

Methods and Materials

Surface sterilized tomato seeds (Lycopersicon esculentum, cv. Vendor) were pregerminated on potato dextrose agar for 5 d, and seeded in coarse sand on a layer of leak roots mycorrhized with Glomus #3 endogenous on ash. After 4 wk, they were placed in 18-cm pots (one per pot) containing either one of the five pasteurized substrates under test: Turface® (T), Turface-Vermiculite (T:V, 1:1), peat moss-Vermiculite-coarse sand (Pm:V:S, 2:1:1), peat moss-Vermiculite (Pm:V, 1:1) and peat moss-coarse sand (Pm:S, 1:1). Roots were inoculated with 1 mL of a Fusarium oxysporum f.sp. radicis-lycopersici suspension (7.0×10^4 propagules [ppg]/mL). The controls received 1 mL of sterilized distilled water. The treatments were: mycorrhizae only (M+F-), Fusarium only (M-F+), mycorrhizae and Fusarium (M+F+), and neither one (M-F-), and were replicated five times per substrate. The plants were kept in a greenhouse for 7 wk, watered with demineralized distilled water, and received 4 mg P/wk.

Humidity (65°C, 24h), salinity (conductivity), pH and available P (Bray II) were determined for each substrate prior to and at the end of the experiment. For each treatment, dry matter yield, % root necrosis, number of Fusarium ppg/g (dry weight) of substrate, and root endomycorrhizal colonization (REC) index based on 40 x 1-cm root pieces per plant were measured. The experimental design was a 5 blocks split plot with 4 treatments randomized among the main plot and 5 substrates randomized among the subplots.

Results and Discussion

There was no pH variation observed after 7 wk (Table 1). Turface alone or in mixture with Vermiculite had a pH close to neutrality. Only Turface provided stable and sufficient humidity. The salinity of Pm:S was the only one classified as good, while average for Pm:V:S, tolerable for T and T:V, and excessive for Pm:V. Non mycorrhized plants grown in T and T:V showed no symptoms of P deficiency, thereby providing reliable controls.

Table 1. Substrate conditions prior to and at the end of the experiment.

	Conditions							
Sub-	pH		Humidity (%)		Salinity (mS/cm)		Available P (ppm)	
strate	i†	f§	i	f	i	f	i	f
T	7.3	7.5	31.0	32.1	1.64	1.42	90	151
T:V	7.6	7.6	37.8	29.5	1.50	1.18	85	139
Pm:V:S	6.1	5.7	42.3	19.3	1.74	1.00	17	31
Pm:V	4.6	4.8	37.3	58.7	5.35	6.68	60	35
Pm:S	5.6	5.6	28.3	8.1	0.26	0.55	50	35

†:initial §:final

The use of Turface has provided the best mycorrhization (Table 2). The presence of Glomus #3 decreased root necrosis, indicating a protective effect. A decrease in the number of ppg of Fusarium in the presence of Glomus #3 was more evident in T and T:V and slightly noticeable in Pm:V:S and Pm:S. Although tomatoes were less infected, the presence of Glomus #3 increased the number of ppg of Fusarium in Pm:V. There was no interaction between substrates and treatments for dry matter yield.

Table 2. Effect of substrate and inoculation on mycorrhization, root necrosis and Fusarium population.

	REC index (%)		Root necrosis (%)		Fusarium (ppg)	
Sub-						
strate	M+F-	M+F+	M+F+	M-F+	M+F+	M-F+
T	42.0a†	21.8a	9.2a	31.7a	154b	3426b
T:V	24.0a	17.9a	12.3a	28.7a	325b	3722b
Pm:V:S	3.9c	2.1c	15.7a	24.7a	1095b	3387b
Pm:V	6.8b	7.4b	17.9a	36.6a	38 288a	22 412a
Pm:S	9.5b	7.4b	12.7a	30.3a	2647b	3300b

†:Means within a column not sharing a common letter differ significantly (P=0.05) by Duncan test.

While providing good experimental conditions, the use of Turface facilitated washing, recuperation, necrosis evaluation and staining of roots. Plenchette et al. (1982) have described the use of Turface to minimize the effect of soil conditions in endomycorrhizal research. Great variation in mycorrhization of tomatoes and fungal pathogen ppg was observed between substrates. One of them, Turface, offers great potential for interactions studies and comparisons between similar or different VAM-host-pathogenic fungus system.

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THE ANNUAL DYNAMIC OF VA-MYCORRHIZAE IN A MARITIME SANDY DUNE (CENTRAL EAST ITALY)

by
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Keywords:ecology,Glomus,Gigaspora,Ammophila litoralis,ammophilous vegetation.

Introduction

Endogonaceous fungi and mycorrhiza VA are wide spread in plant communities in sand soil.However, the correlation of their biology with environmental factors does not seem to be completely understood in spite of previous studies (Koske & Halvorson,1981). In addition,the diffusion of fungal endophytes in natural sites in the Mediterranean area has only recently been studied .(Giovannetti & Nicolson,1983; Dodd & Krikun,1984).
The aim of the present study was to fill in these gaps.

Methods and Materials

A littoral dune on the Adriatic coast near Campomarino (Molise) has been sampled every month, since March 1983,working on transepts parallel to the coast from the beginning of the vegetation as far as the maquis. It is a strip of about 30-40 meters with ammophilous vegetation first with items of Kakiletea maritimae and then of Ammophiletum arundinaceae together with transgressive entities from Agropyretum mediterraneum.
The dominating marine winds from the NE prevent the clotting phase.
The sandy substratum is decidedly basic (pH 9.3-10). The critical factor for the setting-up of vegetation seems to be electrical conducibility (EC) and then osmotic pressure.
10 samples were carried out every 10 mt.collecting plant roots and surrounding sand. The grid-line intersect method was used to estimate the percentage of mycorrhizal infection on stained roots. Endogonaceous spores from 100gr. d.w. of soil were recovered by wet-sieving on 400,250,100 and 40 um nylon sieves.

Results and Discussion

The mycorrhiza VA is diffused in almost all plants supported by five species of Endogonaceae: Glomus deserticola,G.geosporum,G.mosseae,Glomus nov.sp.,Gigaspora gregaria and G.heterogama.
The presence of an ectomycorrhizal fungus like Coenococcum geophilum probably coming from a remote reafforestation with Pinus halepensis has also been irregularly raised.

During the first year's research,we studied the dynamic of endophytes particularly on the rhizosphere of Ammophila litoralis,which was plentifull in each transept. The results are shown in fig.1 with the presence of spores in two annual maxima (on March and July 1983) and their almost complete disappearance during the winter months (from November through January),particularly evident in the first two sample lines (transept 1 and 2).
The spores were more numerous in the external stripe than in the inner one. The mycorrhizal percentage was rather low (10-30%ca) and difficult to interpret. The survey of infected roots of Ammophila litoralis presented some methodological problems which have only been solved recently.
The production of spores seems to be connected more with the climate,and in particular with precipitations,than with phenology or with the species cycle. The two greatest amounts of spores come in periods of drought after rain. This seems to be the opposite of what appears in the literature for the requirement of ectomycorrhizal fungi fructification in which the sporocarps are said to occur in wet periods after periods of drough (Lange,1948)

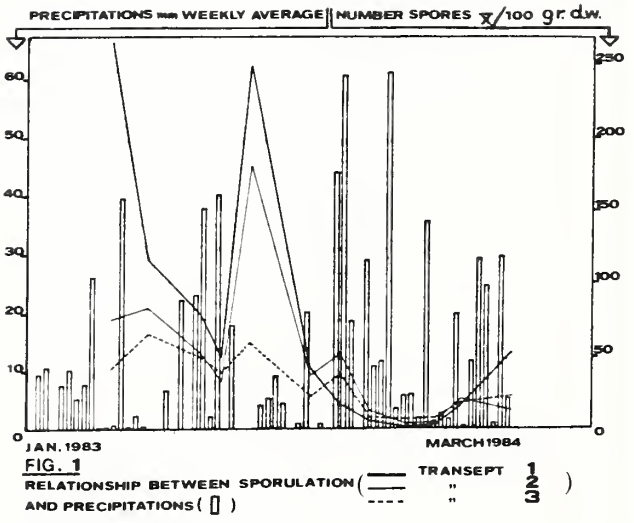

FIG. 1
RELATIONSHIP BETWEEN SPORULATION AND PRECIPITATIONS

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THE ROLE OF V-A-M IN HIGH PHOSPHORUS SORBING SOILS

By

J. Krikun, J. Haas and B. Bar-Joseph

Keywords--soil fumigation, trickle fertigation, phosphoric acid, V-A-M soil inoculation

Introduction

In Israel, due to the rapid buildup of soilborne pathogens under a number of cropping systems, soil fumigation is routinely practised. In most cases, this measure greatly alleviates yield losses, but in a number of crops severe stunting has been observed following treatment with methyl bromide or metham sodium, two highly effective biocides. The studies reported here were undertaken in order to determine: 1. if the stunting was due to lack of V-A-M infection and, if so, could increased rates of superphosphate overcome their absence. 2. if other methods of supplying P would be more effective than the above. 3. if the reintroduction of V-A-M inoculum would overcome the symptoms observed.

Methods and Materials

Two experiments were conducted. In one, carried out on a loessial soil (pH 7.8; 20% $CaCO_3$) the effect of fumigation and superphosphate additions was studied on four crops: pepper, onion, celery and melon. The first three species were suspected of being dependent on V-A-M infection; the last, based on field observations, was deemed not to be dependent. Plots with 0, 1,000, 2,000 and 3,000 kg ha^{-1} superphosphate were established. Half of the plots were fumigated with 500 kg ha^{-1} methyl bromide; unfumigated plots served as mycorrhizal controls. All crops except onions were introduced as transplants. Observations were made as to: V-A-M infection; plant growth; yield and tissue P content. There were four replications.

In experiment two, carried out in a landfill soil (pH 8.1, 50% $CaCO_3$) the effect of phosphorus additions delivered *via* the trickle irrigation system and added as phosphoric acid was evaluated on pepper, the major crop in the region. The effect of adding V-A-M inoculum was also evaluated. Fumigation was as in experiment one; planting was carried out by sowing. Three P concentrations for the first two months of the growing period were evaluated. These were 1, 15 and 40 PPM P applied at each irrigation which was carried out every two days. After this time period all treatments received 20 PPM P with every irrigation. Data was obtained on plant growth, yield, distribution of yield according to size and tissue macroelement composition. There were five replications.

Results and Discussion

In both experiments examination of the roots showed that the fumigation killed the indigenous V-A-M populations. In experiment one, pepper, onion and celery were severely stunted and in essence produced no marketable yield; the phosphorus additions failed to overcome the lack infection. Yields in the non-fumigated plots were excellent, and the roots were mycorrhizal. Melon, in contrast, grew extremely well in all plots and at the highest P addition (3,000 kg ha^{-1}) produced a higher yield in the fumigated plots as compared to the non-fumigated ones. This observation has often been made in commercial plantings.

Tissue analysis of the stunted crops showed that they were extremely deficient in P, non-mycorrhizal plants having half the concentration of mycorrhizal ones. In melon such differences were not noted. Soil P analysis showed that the addition of superphosphate did not greatly increase available P. This was especially true when water was used as the extractant.

In experiment two, it was shown that moderate to high P additions, supplied *via* the irrigation water could overcome, to a large extent, the stunting known to occur on pepper under these conditions. The addition of V-A-M had even more dramatic effects. In this case both total yield and percent of large fruit was increased even when high rates of P were supplied as phosphoric acid.

The results from the experiments presented here, and in other carried out previously, point out that a number of crop species growing in moderately to high P sorbing soils are extremely dependent on the mycorrhizal association for P uptake and normal development. We also believe, based on previous results (1), that the V-A-M populations in these soils are extremely efficient. Until the problem of supplying high quality inoculum is overcome, pallative measures, such as supplying P as phosphoric acid offer a reasonable solution to some crops under our conditions. However, the final aim should be the addition of high quality inoculum, as we believe that the mycorrhizal symbiosis benefits plant growth by other mechanisms besides P uptake.

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HOST-PLANT RESISTANCE TO INSECT PESTS ALTERED BY GLOMUS FASCICULATUM COLONIZATION

R. S. Pacovsky, L. B. Rabin, C. B. Montllor and A. C. Waiss, Jr.

Keywords--Soybean, bollworm, *Heliothis zea*, fall armyworm, *Spodoptera frugiperda*

Introduction

The large-scale cultivation of soybean in the United States has encouraged the establishment of a number of insect pests that cause significant crop damage and economic loss. Presently, concentrated insecticides are being used to control this problem. The suggestion that prior infection of a plant by a pathogen increases a plant's resistance to a second pest may be the basis of a new biological control strategy. Infection by vesicular-arbuscular mycorrhizal (VAM) fungi elicits a host-plant response similar to that of a pathogen. The purpose of this study was to determine if VAM-colonized soybeans would alter susceptibility to two soybean pests.

Materials and Methods

Four soybean (*Glycine max* L. Merr) cultivars, two resistant to several insect pests (PI227687 and PI 229358) and two susceptible (Davis and Amsoy 71) were used. Plants were either inoculated with the VAM fungus *Glomus fasciculatum* (Thaxt *sensu* Gerd.) or were left uninoculated and fertilized with 0.2 mM P. Plants were raised in a growth chamber and harvested over a 3 week period at 8 to 11 weeks of age. Leaves were detached from single plants over a 3 day period and fed to the neonate lepidopteran larvae *Heliothis zea* (Boddie) and *Spodoptera frugiperda* (Smith). Insects were fed fresh leaves daily and some leaves were frozen in liquid N_2 and lyophilized for chemical analyses. Roots were assessed for VAM-colonization and biomass. For each cultivar, there were seven soybean plants and twelve insect larvae per treatment.

Results and Discussion

Table 1. Developmental differences for insect pests fed P-fertilized or VAM soybean leaves. 1/

Cultivar	Larval Weight (14 day)	Pupal Weight	Days to Pupation	Mortality
	%			
	Heliothis zea			
PI227687	-45*	-7	+26*	+25*
PI229358	-30	-24*	+49*	+ 8
Davis	-56*	-30*	+20*	+ 9
Amsoy 71	-63*	-16*	+31*	+17*
MEAN	-49	-19	+32	+15
	Spodoptera frugiperda			
PI227687	-43*	-23*	+10*	- 9
PI229358	-56*	-29*	+17*	+25*
Davis	-33*	+ 6	+ 8	0
Amsoy 71	-44*	ND	ND	ND
MEAN	-43	-15	+12	+ 6

1/Values (% differences) were calculated as: ([VAM - P]/P) x 100. ND = no data

*indicates significant difference at $p < 0.05$.

The average larval weights for *H. zea* and *S. frugiperda* fed leaves from VAM plants were reduced by more than 40% compared to larvae fed P-fertilized plant foliage (Table 1). When fed VAM plants the larvae of both species took longer to pupate, the average pupal weight was 17% lower, and the mortality of *H. zea* was 15% greater than that of the P-fertilized controls. Growth reduction was greatest for *H. zea* in the suseptible cultivars and greatest for *S. frugiperda* in the resistant cultivars that were fed leaves from VAM-infected plants (Table 1).

Growth reduction for larvae fed VAM leaves did not correlate with leaf N, amino acid, carbohydrate, micronutrient, or phenolic content. Dry weights of P-fertilized plants were 25% greater than VAM plants, but they contained 50% more P. However, the decrease in insect growth was not well correlated with the lower P content of VAM plants, and it is unlikely this effect was due to a P deficiency.

Colonization by VAM fungi differed between the four soybean cultivars (Table 2). Percent fungal infection was correlated with differences in larval growth ($r = 0.73$), maturation ($r = 0.88$), and mortality ($r = 0.96$) for combined insect data. This suggests the possibility of a correlation between the plant's response to VAM-fungal infection and subsequent altered resistance to these insect pests.

Table 2. VAM fungal colonization of the four soybean cultivars. 2/

Cultivar	Infection	% VAM biomass in mycorrhizae
	%	%
PI227687	59 bc	5.2 b
PI229358	67 ab	5.7 b
Davis	53 c	4.1 c
Amsoy	71 a	6.8 a

2/Mean values having a common letter are not significantly different at the 0.05 level.

Conclusions

The VAM-induced decrease in insect growth was evident in all 4 cultivars tested. Possible explanations for this effect include altered nutrient content, the presence of a toxin or anti-feedant, or the induction of a general plant resistance mechanism. The VAM-induced decrease in insect growth may be specific for larvae which are foliar feeders since we observed no difference in the feeding or reproductive behavior of a phloem-feeding aphid (*Schizaphis graminum*) tested on VAM-colonized or P-fertilized sorghum. We cannot yet determine whether this effect is due to differences in the host, insect or both.

The growth and maturation of individual lepidopteran insects was altered when they ingested foliage from VAM-infected soybeans. If this effect was generalized to an entire insect population and resulted in lower numbers and viability, then there might be less damage and economic loss in soybeans.

SOIL pH AND VESICULAR-ARBUSCULAR MYCORRHIZA

By

Gamin Wang, D.P. Stribley, P.B. Tinker & C. Walker

Keywords--Glomus tenue, G. caledonicum, fine endophyte, coarse endophyte, aluminium, manganese

Introduction

The effects of soil pH on natural populations of vesicular-arbuscular (VA) fungi have not been studied systematically. There is little information on the occurrence of species in relation to pH (Mosse, Stribley & LeTacon, 1981). A useful opportunity for a study specifically on pH is afforded by the long-term liming experiments at Rothamsted Experimental Station where two sites, initially uniform, have been maintained at four levels of pH (ca, 4.5, 5.5, 6.5 and 7.5 respectively) for 22 years by differential liming (Bolton 1977). We present observations on the mycorrhizal fungi of these sites, and investigations on the mechanism of the pH effects.

Methods and Materials

Mycorrhizal fungi in the long-term liming experiment. These experiments were sown to spring oats ('Peniarth') in 1981 and 1982, and to maincrop potatoes ('Pentland Crown') in 1983. Root samples from plots of each pH were taken every year with a corer to 15 cm depth, the roots washed out, then cleared and stained. The fractional infection by mycorrhiza was measured by a grid-intersect method.

Effects of additions of Calcium carbonate. Finely ground $CaCO_3$ was added to field soil from the most acid plots at Rothamsted, to give soils of pH 4.5, 5.5, 6.5 and 7.5 respectively. Spring oat was grown on these soils in pots in the glasshouse and the ratio of 'fine' (Glomus tenue) and 'coarse' endophtes in the roots measured after 15 weeks.

Effects of aluminium and manganese in sand culture. Winter oat ('Pennal') was grown on acid-washed sand, with a basal nutrient supply, at four levels of Al, or four levels of Mn, respectively at pH 4.5. The inoculum was a laboratory culture of G. caledonicum maintained in soil of pH 7.5 with leek (Allium porrum L.) as host. Fraction infection was measured at 10 weeks.

Results and Discussion

pH and VA fungi in the long-term liming experiments. Figure 1 shows that roots on acid soil in the field were exclusively colonized by fine endophyte and that the proportion fine:coarse endophytes decreased with increasing pH. These results were consistent throughout three seasons, and were similar for potato. Overall levels of infection at harvest were unaffected by pH. This is consistent with the result of Read, Koucheki & Hodgson (1976) who found a constant level of infection in the perennial grass Festuca ovina L. over the pH range 4.2-7.0. Similarly, Sparling & Tinker (1978) observed little effect of pH in the range 4.9 to 6.2 in three grassland sites.

Effects of sudden change in pH. When soil from the acid plots was limed in a pot experiment, fine endophyte infected well at low pH but not at high pH, as in the field. Coarse endophytes had apparently survived in the acid soil and their spread was favoured by increased pH.

Effects of aluminium and manganese. Figure 2 shows that mycorrhizal infection by a stock isolate of G. caledonicum was inhibited strongly be aluminium in solution culture. Manganese was about five times less inhibitory. Low pH per se did not inhibit infection, providing these toxic metals were absent. The levels of aluminium used in sand culture were similar to those in soil solution of field soil.

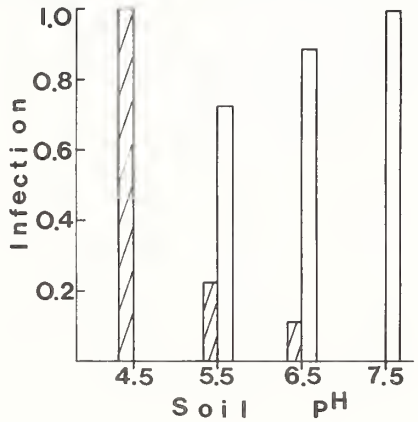

Figure 1. Proportions of fractional infection by fine (▨) and coarse (□) endophytes in roots of field-grown oat.

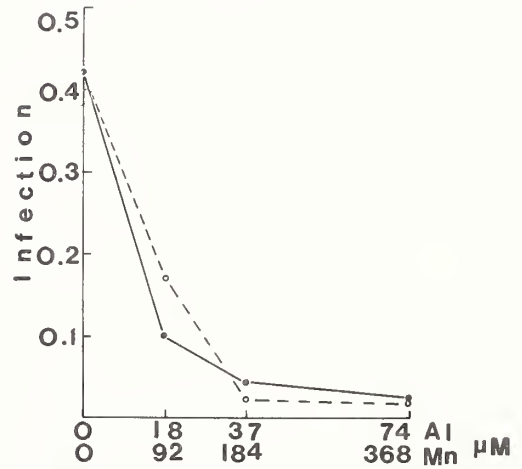

Figure 2. Effects of Al (● - ●) and Mn (o--o) in sand culture on fractional infection by G. caledonicum at pH 4.5.

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COMPARATIVE WATER RELATIONS OF VA MYCORRHIZAL AND NONMYCORRHIZAL GRASSES ON DISTURBED LAND

By

E. B. Allen, and M. F. Allen. Dept. Range Science and Dept. Biology. Utah State Univeristy, Logan, Utah.

Keywords--Agropyron dasystachyum, A. smithii, stomatal resistance, water potential

Introduction

Studies showing increased water movement through mycorrhizal plants have been restricted to greenhouse pots (Safir et al. 1972, Allen et al. 1981). This may be due to the difficulty of establishing nonmycorrhizal control plants in the field, as well as uncontrolled factors such as competition from neighboring plants. A field study was designed to show the effects of both mycorrhizae and colonizing annuals on water relations of Agropyron smithii and A. dasystachyum.

Methods

The study site was at a coal stripmine in SW Wyoming which receives 23 cm precipitation but had 70 cm during the study year. Selected plots were inoculated with 1-3 cm fresh topsoil. Planted grasses had 20% infection on inoculated quadrats and 6% on uninoculated quadrats. Colonizing annuals, mainly nonmycotrophic species in the Chenopodiaceae, were removed by hand from one half of the plots in both inoculum treatments. Soil and grass leaf water potentials and stomatal resistance (r_s) were monitored.

Results and Discussion

There were no changes in % cover of the grasses due to mycorrhizae or annuals during this wet year. Inoculated A. smithii had lower r_s than uninoculated plants, but only when soils were relatively dry. This indicates that mycorrhizae may be more beneficial for increased water transport in a dry soil.

There were no significant changes in r_s of inoculated A. smithii with or without annuals, but the uninoculated plants had greater r_s when annuals were present than absent (Fig. 1). This suggests that mycorrhizae alleviate the negative effects of competition from annuals on r_s of A. smithii.

Finally, A. dasystachyum had no changes in r_s due to inoculation, suggesting that it is not as dependent as A. smithii on mycorrhizae for increased survival in an arid climate.

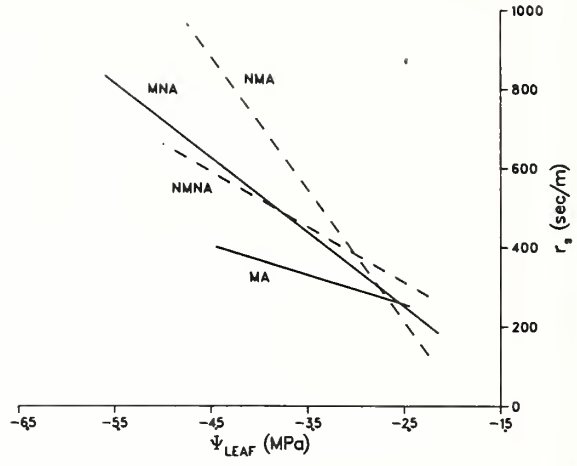

Fig. 1. Stomatal resistance of A. smithii vs. leaf water potentials measured at midday throughout the growing season. M = with mycorrhizal inoculum. NM = no mycorrhizal inoculum. A = with annual plants. NA = no annuals. The following table gives 2x2 t-test comparisons among slopes (S) and elevations (E). n.s. = not significant. *, ** = significant at 0.95, 0.99 probability levels. Elevations significant at leaf water potential $\leq$ -3.8 MPa.

TREATMENT	MNA	MA	NMNA
MA	S 0.32 n.s. E 1.38 n.s.	____	____
NMNA	S 0.76 n.s. E 2.99 **	0.79 n.s. 4.96 **	____
NMA	S 2.23 *	3.32 **	2.23 **

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ECESIS ON MOUNT ST. HELENS: CAN MYCORRHIZAL FUNGI SPREAD FROM ANIMAL-DISPERSED INOCULUM?

By

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Keywords--Glomus, Endogone, gophers, ecesis

Introduction

In May 1980, Mount St. Helens erupted disturbing over 350 km^2 of forest and montane habitats. In several locations, gophers survived by remaining below ground. Following ash deposition, they tunnelled to the surface bringing up old soil containing plant propagules and mycorrhizal fungi. Our objective is to determine if these gopher mounds can serve as islands to initiate succession.

Null Hypotheses

1. There are no differences in VAM fungal spores counts in fresh gopher mounds and adjacent tephra.

2. There are no differences in VAM infection in plants growing in gopher mounds and adjacent tephra.

3. With time there is no migration of VAM fungal spores out onto the tephra.

Methods

Spores were separated from appropriate soils using differential centrifugation. Root infection was estimated by staining the line intercept. Soils were dried at 90° C for 48 hours and counts expressed on a dry soil mass basis.

Results

1. Glomus and Endogone spore counts were higher in mound than nonmound material at all sites except the Pumice plain where the old soil could not be reached (tephra 20 m deep), (Table 1).

2. VAM infection was limited to plants growing on gopher mounds or with roots extending into those mounds (Table 1).

3. With time, Glomus spores migrated out onto tephra material. Most Endogone spores apparently disappeared (Fig. 2).

Conclusion

Gophers distribute seeds and VAM inoculum to the surface of the tephra. These mounds appear to act as focal points of community establishment initiating succession.

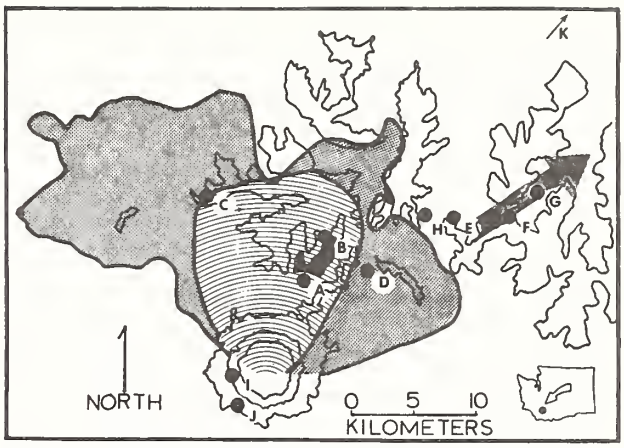

Fig. 1. Sample locations of mound and adjacent nonmound tephra at 2 types of sites; where gophers naturally survived (sites B, D, E, F, G, H, J, K) and where gophers were introduced in 1 m^2 mesh enclosures for 24 h (sites A, C, I).

Table 1. Root infection and spore counts (Endogonaceae) in high tephra fall zones.

Plant Species	Soil Type	Root Infection (% Root length)	Spore Counts (#/g Dry Soil)
Epilobium angustifolium	tephra	0	0
	gopher mound	60	4.5
	old soil	12	28
Anaphalis margaritaceae	tephra	0	0
	gopher mound	72	3
	old soil	n.r.	16
Lupinus latifolius	tephra	0	0
	gopher mound	88	3
	old soil	n.r.	16

n.r. = no roots found.

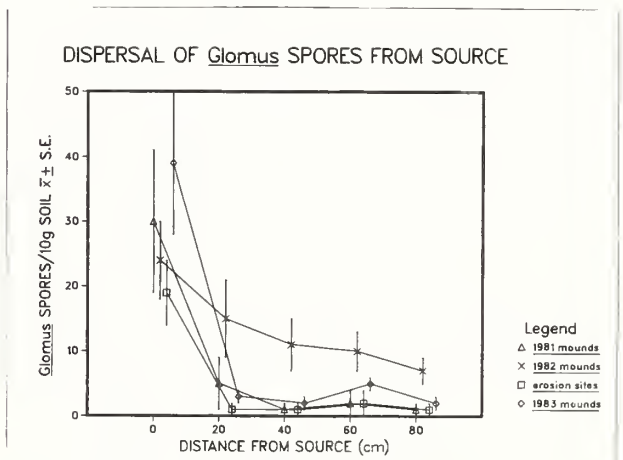

Fig. 2. Dispersal of Glomus spores from source.

DISPERSAL OF VA-MYCORRHIZAL FUNGI IN A DISTURBED SEMI-ARID ECOSYSTEM

By

N. Warner, J. A. MacMahon and M. F. Allen

Keywords--wind, grasshoppers, rodents, Glomus spores, dispersal, strip-mine

Introduction

Studies were conducted to evaluate the potential roles of wind, small mammals and grasshoppers in the dispersal of VA-mycorrhial spores from an undisturbed area to an area disturbed by strip-mining. It is hypothesized that small mammals, grasshoppers and wind are not dispersal agents of Endogonaceae spores, and that spore densities do not decrease as distance from an undisturbed source increases.

Methods and Materials

Rodents, grasshoppers and air samples were collected on a recontoured and retopsoiled strip-mine and adjacent undisturbed areas, followed by microscopic inspection for spores. Also, a series of plots were established on the disturbed site where mammals were excluded, mammals and grasshoppers were excluded and no animals were excluded, or controls. Soil samples were removed from these plots and spores were extracted and counted at 30X. Sampling was conducted during the spring, summer and fall of 1982-3.

Rodents were trapped, identified and a fecal sample was collected prior to their release. Grasshoppers were captured, held in vials until they defecated and killed. Air samples were obtained through the use of 40 spore traps (Fig. 1) arranged in 2 transects originating in the undisturbed area and extending 55 m up the windward slope of the disturbed area.

Results and Discussion

All spores isolated in this study were species of Glomus. VA-mycorrhizal spores were recovered from the feces of 27% of the rodents captured on the disturbed and adjacent undisturbed area. 31% and 3% of the grasshoppers captured in 1982 and 1983 had spores on the exterior body surface or in the feces and gut contents. More spores were captured with the spore traps in 1982 than in 1983. The greater number of spores isolated from grasshoppers and captured with the spore traps in 1982 may be due to a decrease in spore density on both disturbed and undisturbed areas in 1983. This decrease may have been influenced by the unusually wet conditions during sporulation in the fall of 1982.

Soil sampling of exclosure and control plots did not reveal any significant differences in mean spore counts between treatments in 1982-3. There was a significant effect due to season (Fig. 2) which may reflect the decomposition of residual spores in 1982 and their gradual recolonization in 1983. Mean spore counts were greater at sites further from the undisturbed area than at adjacent sites (Fig. 3).

It is concluded that rodents, grasshoppers and wind are dispersal agents of VA-mycorrhizal spores in the environment studied. This study shows that spore densities do not necessarily decrease as distance from a source area increases.

Figure 1. Spore trap used in air sampling.

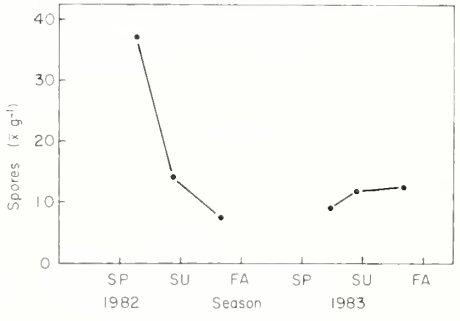

Figure 2. Change in mean VAM spore density from exclosure and control plots on the disturbed area.

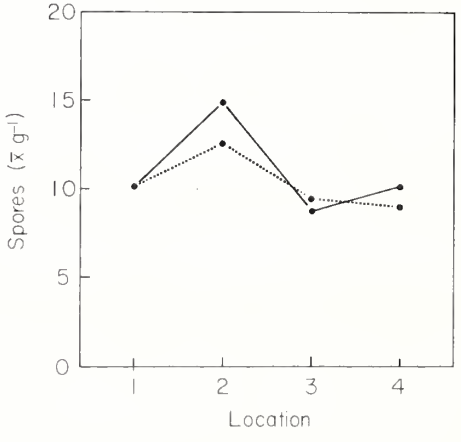

Figure 3. Mean VAM spore counts from exclosure and control plots at locations increasingly distant from the undisturbed area. — = all seasons, --- = spring and summer only.

"RELATIVE PRODUCTIVITY" OF MYCORRHIZAL FUNGI, MYCORRHIZAE AND CLIMATIC FACTORS.

By

R. Agerer

Keywords: Vorausgangs-Precipitations, weekly mean temperature, Picea abies, Quercus robur, Fagus silvatica, Russulaceae, Cortinariaceae.

Introduction

It is obvious that growth of fruitbodies of mycorrhizal fungi is dependent upon climatic factors on the one hand and on the other upon mycorrhizae. We tried to find out whether there are direct correlations between volume-equivalents of fruitbodies and climatic factors resp. mycorrhizae.

Material and Methods

For estimating the volume-equivalents of fruitbodies we used the "relative productivity" as it was done in Agerer & Kottke (1981). We compared the "relative productivity" (i) with the so-called "Vorausgangs-Precipitations"; this term means those precipitations which have fallen for a certain number of weeks till any time of the year - in this case we summed up the precipitations of seven weeks -, (ii) with the weekly mean temperature, (iii) with the number of mycorrhizae per soil volume and with some additional climatic dates. The results of this study are based on four-year researches of three different plots in the "Naturpark Schönbuch" in the southwest of Western Germany. The plots were fenced, 1200 squaremetres in size and they are deviating from one another either in soil characteristics or in the stock of trees. Two plots with spruce (Picea abies) - one with sandy the other with silty clay soil - and one plot with deciduous trees (Fagus silvatica, Quercus robur) with silty clay soil were investigated.

Results and Discussion

We were able to demonstrate that for growing of fruitbodies of the mycorrhizal fungi and for growing of mycorrhizae the weekly mean temperature in the first half of the year is very important. Another factor, but obviously not as important as the temperature, are the so-called "Vorausgangs-Precipitations".

It seems that the distances between the maxima of weekly mean temperature in the first half of the year would determine the distances between the maxima of relative productivity of mycorrhizal fungi. Smaller distances between maxima of weekly mean temperature in the first half of the year result in smaller distances between the maxima of relative productivity. Which maximum of the weekly mean temperature of the existing two or three maxima in the first half of the year entails the first maximum of relative productivity of mycorrhizal fungi is probably depending on the stock of trees (coniferous or deciduous trees). The highest maximum of weekly mean temperature always corresponds with the highest maximum of relative productivity.

For one of the plots (spruce on sandy soil) we could demonstrate that, if the sum of centigrades above the zero-centigrade-line is greater in comparison with such of other years then the maxima of relative productivity will appear earlier. Probably there is a correlation between the size of this sum and the number of weeks, which will pass till the appearance of the maxima of relative productivity.

There exists a close correlation, too, between the maxima of relative productivity and the graphs of mycorrhizae per soil volume; this is true especially in dry years.

As the maxima of relative productivity correspond with the weekly mean temperature maxima of the first half of the year on the one hand and with the mycorrhizae per soil volume on the other, the mycorrhizae in the season of fructification are probably due to the temperature of the first half to the year. Further it could be shown, that the early weekly mean temperature maxima of the first half of the year - this is true at least for all plots in the one and only year in which this problem was studied - make mycorrhizae increase their number per soil volume, just at that point of time.

The "Vorausgangs-Precipitations" are able to modify the maxima of relative productivity in shape and size.

The growth of mycorrhizae early in the year seems rather to be dependent on the weekly mean temperature than on the "Vorausgangs-Precipitations". And the number of mycorrhizae in the season of fructification is influenced not only by the temperature of the first half of the year but also by the "Vorausgangs-Precipitations". They are able to influence the number of mycorrhizae, and probably they only modify the shape of maxima of mycorrhizae in the same way as they influenced the relative productivity.

Depending on the plots (trees, soil) certain relationships of mycorrhizal fungi play an important role for the number of mycorrhizae per soil volume. Especially in dry season these relationships may be responsible almost exclusively for the additionally grown mycorrhizae in the season of fructification. This could be shown especially for the Russulaceae. A similar role play the Cortinariaceae. For one plot (spruce on silty clay) the importance of the genus Hygrophorus (with the species Hygrophorus pustulatus) is obvious. It seems therefore, that mainly these relationships are essential for the growth of trees in these plots at least in dry seasons.

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INTERSPECIFIC COMPETITION BETWEEN GLOMUS MOSSEAE AND GIGASPORA MARGARITA

By

K. L. LaBounty and J.A. Menge

Keywords--interspecific interactions, salinity, Allium cepa

Introduction

Concurrent root infections by several vesicular-arbuscular (VAM) fungi are frequent in natural ecosystems and in agricultural situations (Bethlenfalvay et al., 1984; Koske and Halvorson, 1981; Nemec et al., 1981).

Possible interspecific interactions between VAM species have been reported by Daft and Hogarth, 1983, and Wilson and Trinick, 1983. Since VAM species differ in their effectiveness at increasing nutrient uptake and plant growth, interactions between VAM species could lead to decreased host benefits.

The purpose of this research was to determine if interaction occurred between Glomus mosseae and Gigaspora margarita and if this interaction could be altered by irrigation with a salt solution. The effect of interspecific interaction on the host plant was also investigated.

Methods and Materials

Inoculum consisting of soil, root pieces, and spores was prepared from pot cultures of Glomus mosseae and Gigaspora margarita. Populations of infective propagules in the inoculum were determined using the Most Probable Number technique (MPN). A replacement series was utilized with the following species proportions: (G. mosseae : G. margarita) 0:1; 0:1; 1:1; 1:3; 3:1. Total number of propagules per pot was 50. Inoculum was mixed in autoclaved soil and placed in pine seedling tubes. Either a saline solution (50% NaCl, 35% $CaCl_2$, 15% $MgCl_2 \cdot 6H_2O$) with electroconductivity of 5 mmhos/cm plus 40 ppm $NaNO_3$ or $NaNO_3$ alone was used to irrigate onion plants.

Seven plants were harvested per treatment 7, 14, and 18 weeks after planting. Tops were oven dried at 65 C and weighed. Roots were cleared and stained to determine mycorrhizal colonization for each species. Post experiment inoculum potential was determined for each species using the MPN technique.

Results and Discussion

G. margarita colonized roots at significantly higher rates than G. mosseae at all harvests, whether inoculated singly or in mixtures. G. margarita infected at similar levels in the species mixtures and the single species treatment. G. mosseae colonized the root system at significantly higher levels when used as single species inoculum than in the mixtures.

The salt irrigation significantly decreased dry weight and percent infection at the second and third harvests, but did not alter the interaction between the two species.

Dry weight of plants were significantly greater when plants were inoculated with G. margarita or species mixtures than with G. mosseae or non-inoculated controls.

The post experiment inoculum potential of G. margarita was higher than that of G. mosseae in all of the species mixtures, but were equivalent in the nonsalt treatments when inoculated singly.

G. margarita competes with G. mosseae on onion roots for infection sites. A possible mechanism for this is the rapid colonization and establishment in the root system by G. margarita. The balance between the two species is not affected by salt irrigation, but could be affected by other environmental parameters.

Competition between VAM species could significantly decrease the beneficial effects to the host if the more aggressive species was an ineffective endophyte. Given soil with limiting nutrients and VAM dependent host plants, competition between VAM species could have a role in shaping the structure of plant communities.

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THE ROLE OF VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI IN TALLGRASS PRAIRIE FORBS

By

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Keywords: Babtisia leucantha, Liatris aspera

Introduction

Native prairie plants are resistant to climatic stress and require minimum energy input once established. Adaptation of such species to low-maintenance landscapes is of increasing importance but native forbs are not utilized to their fullest potential because seedling growth is slow and establishment in the field difficult. Direct seeding or transplanting requires knowledge of early growth habit but prairie forbs are long-lived perennials and seedlings are rarely observed probably because of competition from fast-growing grasses. Also probably resulting from competition with grasses, prairie forbs are extremely deep-rooted (20 m or more). The mycorrhizal status of these deep-rooted forbs is unknown and may not be important since mycorrhizal fungi rarely colonize lower soil depths. However, seedling establishment of forbs and their adaptation to low maintenance landscapes may depend on mycorrnizal fungi.

Materials and Methods

Field Data: Prairie soil was collected from 4 sites and VAM species and spore numbers assessed. Prairie forbs were excavated from native prairie, the roots divided into 10 cm sections according to depth and stained to assess VAM root colonization.

Greenhouse Experiment: Seeds of Babtisia leucantha T. & G. (Atlantic wild indigo), Liatris aspera Michx. (rough gayfeather) and Asclepias tuberosa L. (butterfly milkweed) were germinated and transplanted into tubes of 1:1:1 peat:perlite:soil or 1:1 soil: sand, each amended with 0, 41.6, or 83.2 g/cu ft P (superphosphate 0-20-0). Ten replicate tubes were inoculated with G. etunicatum, G. fasciculatum, G. macrocarpum, G. mosseae or remained noninoculated. After 5 months 1/2 of the replications were analyzed for total dry weight and root colonization. The remainder were outplanted to study transplant establishment.

Results and Discussion

Field Data: Analysis of prairie soil revealed moderate numbers of VAM spores with some species variation between sites. Root colonization was heavy even in forb roots growing below 50 cm.

Greenhouse Experiment: Growth of the forb species at 31 ppm P, in 1:1 soil mix was significantly improved by addition of most VAM fungi tested although no one species consistently improved growth of all forbs (Tables 1, 2 & 3). At 688 ppm P, simulating nursery production fertility levels, fewer but occasional VAM growth responses were evident but none was observed at 1762 ppm P, perhaps because here VAM root colonization was reduced. At the low P level in 1:1:1 mix, growth of B. leucantha and A. tuberosa was reduced as compared with growth in 1:1 mix, suggesting that the lower pH of this mix may inhibit plant growth. Of the 3 forbs only B. leucantha benefited from mycorrhizal inoculation in 1:1:1 mix but only at the medium P level. The fact that mycorrhizal colonization of prairie forbs occurs at lower soil depths 'in situ' and also improves seedling growth in the greenhouse suggests that VAM fungi may play an important role in forb establishment and survival in the P-deficient prairie soils as well as highly disturbed areas such as new housing and industrial developments.

Table 1. Influence of phosphorus level and mycorrhizal fungus species on growth of Liatris aspera in two soil mixes.

Fungal species	Total Dry Weight[1] Phosphorus Level Low	Medium	High
1:1 Soil Mix[2]			
Noninoculated	0.09^{g}	0.65^{def}	1.56^{abc}
G. etunicatum	0.26^{g}	1.04^{cde}	0.85^{def}
G. fasciculatum	0.81^{ef}	1.38^{bcd}	0.90^{def}
G. macrocarpum	0.44^{fg}	1.62^{ab}	1.36^{bcd}
G. mosseae	0.79^{ef}	1.24^{bcde}	1.95^{a}
1:1:1 Soil Mix[2]			
Noninoculated	0.10^{z}	0.99^{xy}	1.15^{wxy}
G. etunicatum	0.32^{z}	1.11^{wxy}	1.69^{w}
G. fasciculatum	0.57^{yz}	1.13^{wxy}	1.29^{wx}
G. macrocarpum	0.56^{yz}	1.64^{w}	1.63^{w}
G. mosseae	0.63^{yz}	1.0^{xy}	1.45^{wx}

[1]Means followed by the same letter are not significantly (P=0.05) different as determined by LS Means.

[2]Phosphorus level and pH of Low, Medium and High P treatment in 1:1 soil were 31 ppm P/pH 7.9, 688 ppm P/pH 7.8 and 1762 ppm P/pH 6.5, respectively while 1:1:1 soil contained 40 ppm P/pH 6.6, 700 ppm P/pH 5.8 and 1650 ppm P/pH 5.4, respectively.

Table 2. Influence of phosphorus level and mycorrhizal fungus species on growth of Asclepias tuberosa in two soil mixes.

Fungal Species	Total Dry Weight[1] Phosphorus Level Low	Medium	High
1:1 Soil Mix[2]			
Noninoculated	0.13^{d}	0.42^{cd}	0.55^{bcd}
G. etunicatum	0.66^{abcd}	1.06^{abc}	0.76^{abc}
G. fasciculatum	0.72^{abc}	1.18^{a}	0.62^{abc}
G. macrocarpum	0.97^{abc}	1.12^{ab}	1.13^{ab}
G. mosseae	0.52^{bcd}	0.76^{abc}	0.77^{abc}
1:1:1 Soil Mix[2]			
Noninoculated	0.05^{z}	0.69^{wxyz}	0.51^{xyz}
G. etunicatum	0.38^{xyz}	1.01^{wx}	0.70^{wxyz}
G. fasciculatum	0.33^{yz}	0.85^{wxy}	0.73^{wxy}
G. macrocarpum	0.31^{yz}	0.86^{wxy}	1.18^{w}
G. mosseae	0.23^{yz}	1.07^{wx}	0.96^{wx}

[1]Means followed by the same letter are not significantly (P=0.05) different as determined by LS Means.

[2]Phosphorus level and pH of Low, Medium, and High P treatments in 1:1 soil were 31 ppm P/pH 7.9, 688 ppm P/pH 7.8 and 1762 ppm P/pH 6.5, respectively while 1:1:1 soil contained 40 ppm P/pH 6.6, 700 ppm P/pH 5.8 and 1650 ppm P/pH 5.4, respectively.

Table 3. Influence of phosphorus level and mycorrhizal fungus species on growth of Babtisia leucantha in two soil mixes.

Fungal Species	Total Dry Weight[1] Phosphorus Level Low	Medium	High
1:1 Soil Mix[2]			
Noninoculated	0.47^{f}	1.47^{ab}	1.49^{ab}
G. etunicatum	1.05^{cde}	0.99^{cde}	1.17^{abcd}
G. fasciculatum	0.76^{def}	1.16^{abcd}	1.14^{abcd}
G. macrocarpum	1.03^{cde}	1.12^{abcd}	1.16^{abcd}
G. mosseae	0.98^{cde}	1.30^{abc}	1.72^{a}
1:1:1 Soil Mix[2]			
Noninoculated	0.17^{xy}	0.64^{vwx}	0.24^{vwx}
G. etunicatum	0.60^{vwxy}	1.34^{s}	0.44^{vwxyz}
G. fasciculatum	0.80^{tuv}	0.83^{tuv}	0.36^{wxyz}
G. macrocarpum	0.32^{wxyz}	1.20^{st}	0.07^{z}
G. mosseae	0.66^{uvw}	1.07^{stu}	0.60^{vwx}

[1]Means followed by the same letter are not significantly (P=0.05) different as determined by LS Means.

[2]Phosphorus level and pH of Low, Medium and High P treatments in 1:1 soil were 31 ppm P/pH 7.9, 688 ppm P/pH 7.8 and 1762 ppm P/pH 6.5, respectively while 1:1:1 soil contained 40 ppm P/pH 6.6, 700 ppm P/pH 5.8 and 1650 ppm P/pH 5.4, respectively.

OCCURRENCE OF VA-MYCORRHIZA ON SUGARCANE IN COLOMBIA.

By

S. Toro T., E. Sieverding and C. Castilla C.

Keywords Cultivars, Ecology, Mycorrhizal infection, NPK fertilization, Saccharum officinarum, Soils, Stage of plant growth, VA-mycorrhizal species

Introduction

VA-mycorrhizal infection of sugarcane (S. officinarum) has been reported by Dainese et al (1981) from Brasil and by Huang et al (1982) from Taiwan. Both groups isolated Glomus spp. and Gigaspora spp. from sugarcane fields. In Colombia, no investigation of the occurrence of VA-mycorrhiza on sugarcane was done earlier. Basic research was conducted using field grown sugarcane for the study.

Methods and Materials

For mycorrhizal observation, fine roots from cultivars POJ 28-78 and CP 57-603 were collected from NPK fertilization trials established by CENICAÑA at 4 different sites of the Cauca Valley, Colombia. All fields had been in sugarcane since more than 10 years. Soils were classified as 1) typic pellustert, 6.7 ug P/g; 2) fluvaquentic ustropept, 7 ug P/g; 3) vertic eutropept, 7 ug P/g and 4) pachic haplustoll, 20 ug P/g soil. Organic matter varied between 2 and 3.5%, pH from 5.5 to 6.8. Different combinations of N, P and K were applied in amounts of 0, 50 and 100 kg N/ha; 0, 22 and 44 kg P/ha; and 0, 42 and 84 kg K/ha. Roots were taken at 0-20 cm distance from the plant, up to 15 cm soil depth, during the cane 'boom stage' at 4, 6 and 8 months of age. Indigenous mycorrhizae were multiplied in pots using tropical kudzu as trap plant. After 6 months, mycorrhizal spores were classified using keys of Trappe (1982) and Schenck et al (1984).

Results and Discussion

At all soil sites and in all treatments, both cultivars showed characteristic VA-mycorrhizal infections. Infected root length was influenced by the stage of plant growth, soils and cultivars (Figure 1). Although the overall infection was negatively correlated with the soil P contents, P fertilization only showed a negative influence at site No. 2, when applied alone. K and increasing NPK applications had no effect on mycorrhizal infection. N fertilization alone increased the infection ratings. Importance of N fertilization for infection was confirmed by fertilizer treatments where lack of N reduced the infection. Mycorrhizal species were found of the Endogonaceae genera Glomus, Sclerocystis, Acaulospora, Entrophospora and Gigaspora (Table 1). The occurrence of mycorrhizal species could not be related to site specific conditions. From the 17 morphologicaly different Glomus spp., G. fasciculatum, G. mosseae and G. occultum were identified. From the 2 Acaulospora spp. one was A. appendicula. The unidentified 2 Sclerocystis spp., the Entrophospora sp. and 2 Gigaspora spp., as well as the unidentified Glomus spp. are maintained in pot cultures.

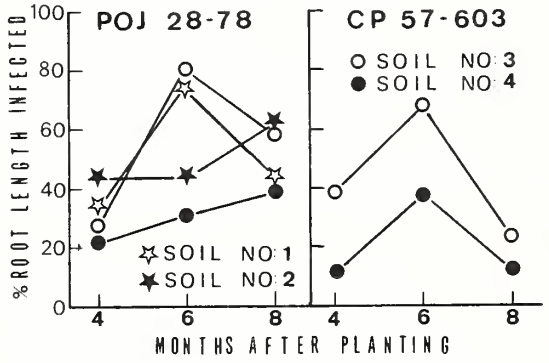

Figure 1. Mycorrhizal infection of 2 sugarcane cultivars in 4 soils (0 fertilizer)

Table 1. Numbers of different species of 5 genera in soils with sugarcane

Soil No.	Glomus	Sclero-cystis	Acaulo-spora	Entropho-spora	Giga-spora
No. 1	7	0	1	0	0
No. 2	5	1	2	0	0
No. 3	9	1	1	1	0
No. 4	13	0	1	1	2

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THE EFFECTS OF TILLAGE TREATMENTS AND A FALLOW SEASON ON VA MYCORRHIZAE OF WINTER WHEAT

By

D. H. Yocom, H. J. Larsen, and M. G. Boosalis

Keywords--Triticum aestivum, Agropyron desertorum, no-till, infection potential

Introduction

Allen and Boosalis (1983) found that two western Nebraska fields in wheat-fallow rotations for over 20 years had significantly fewer Glomus fasciculatum spores than uncultivated grasslands and suggested that continued wheat-fallow rotations can reduce the incidence of VA mycorrhizae. In addition, the current shift from traditional tillage methods to no-till methods in western Nebraska and elsewhere changes soil conditions in a number of ways which may affect mycorrhizal fungi and mycorrhizal interactions. This study is part of a long-term study designed to examine the effects of the wheat-fallow rotation and different tillage methods, including no-till, on the incidence of VA mycorrhizae.

Materials and Methods

Sections of old-growth crested wheatgrass fields at two western Nebraska sites were prepared for wheat planting with one of four tillage treatments: 1) plow, 2) subtill, 3) plow and then chemical fallow (plow/chem), and 4) chemical fallow or no-till (chem). Uncultivated crested wheatgrass sod was maintained as the control treatment. The 4.27 X 9.14 m plots were established in a randomized complete block design with four replicates. The initial fallow period was the 1981 crop year, and winter wheat (Centurk 78) was planted in the treated plots in September, 1981. The plots were sampled periodically over the next two years by taking five randomly located, 15 cm deep soil cores within each plot and pooling the cores as a single sample per plot. The soil samples were processed to determine the field infection levels of the roots, the mycorrhizae infection potential (MIP) by a bioassay procedure, and the density and diversity of mycorrhizal fungal spores.

Results and Discussion

The incidence of mycorrhizal fungi declined significantly during the fallow period (e.g. see the MIP values, Figure 1). This decline was probably due to the lack of host plants to support mycorrhizae, since the crested wheatgrass plots did not show systematic declines in MIP values. Mechanical tillage (plow, subtill, and plow/chem) reduced the incidence of mycorrhizae to a greater degree than the chemical fallow (no-till) method. The total number of mycorrhizal fungal spores declined dramatically at Sidney, and the decline in the incidence of mycorrhizae and mycorrhizal propagules had an effect on the subsequent wheat crop at both locations as seen in the reduced infection of one-month old wheat in 1983 (Table 1). Any failure of mycorrhizal fungus levels to return to the 1981 or 1982 levels during the 1984 wheat crop year would indicate the wheat-fallow cultivation has seriously reduced the abundance of mycorrhizal fungi. A continued erosion of mycorrhizal populations could eventually reduce crop yield.

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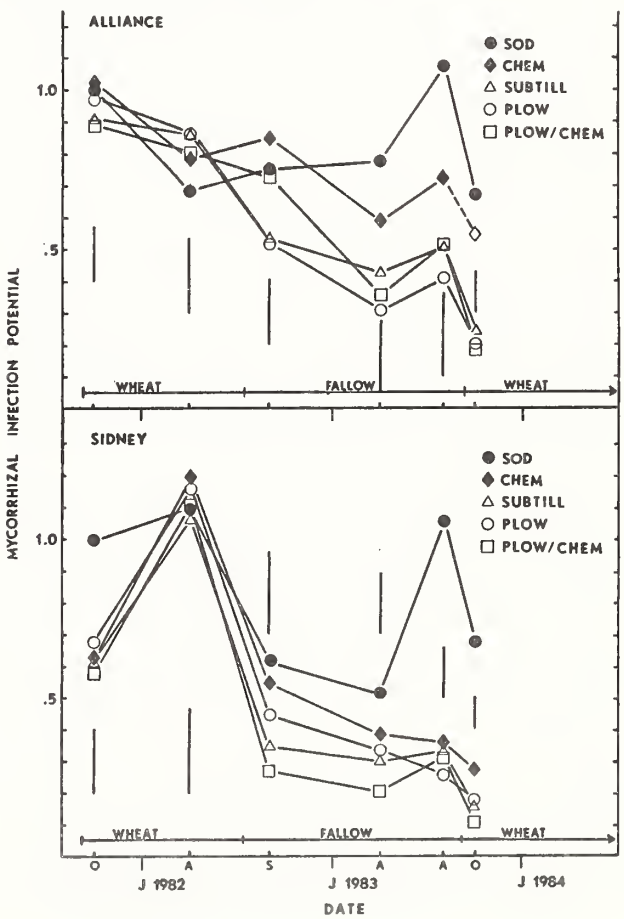
Table 1. Mycorrhizal infection levels of month-old winter wheat at the start of the first wheat-fallow cycle and the start of the second wheat-fallow cycle. Means in a column followed by the same letter are not significantly different (α =0.05).

	Sidney		Alliance	
Treatment	October 1981	October 1983	October 1981	October 1983
Grass sod	34.25 a	41.00 a	37.75 a	40.50 a
Winter wheat				
Chem	25.25 a	25.75 b	25.25 a	no sample
Plow	18.00 a	10.00 c	36.25 a	8.50 b*
Plow/Chem	16.00 a	8.50 c	39.75 a	11.25 b*
Subtill	18.50 a	9.50 c*[1]	40.50 a	11.25 b*

[1] The asterisks indicate a significant difference (α =0.05) between 1981 and 1983 infection values.

Figure 1. Mean mycorrhizae infection potentials (MIP, n=4) for all five treatments on six sampling dates. The vertical bars for each date represent the LSD (α =0.05) for comparisons between treatment means on a single date.



VA MYCORRHIZAE AND HOST PLANT REPRODUCTION: A STUDY WITH GREEN PEPPERS

By

D. H. Yocom

Keywords--Capsicum, Glomus mosseae, fitness, mutualism, phosphorus, reproductive effort

Introduction

The VA mycorrhizal association is usually a mutualistic relationship between a vascular plant and a mycorrhizal fungus (family Endogonaceae). This mutualistic relationship can be described as one in which both organisms receive a net benefit as the result of the interaction. Therefore, each organism should have a greater probability of contributing offspring to the next generation (improved fitness) when interacting with the mutualist than when it exists without the mutualistic interaction. For the VA mycorrhizal fungi, the interaction with host plants seems essential for growth and reproduction, since all efforts to grow the fungi without plants or plant tissue have been unsuccessful. For plants, the vast majority of studies have focused on changes in vegetative growth of plants infected by mycorrhizal fungi. While vegetative growth is often correlated with fitness, a much better correlate is the percentage of the total photosynthetic output (total dry wt.) that is used in reproduction (reproductive effort). This study will compare the reproductive efforts of mycorrhizal and nonmycorrhizal (uninfected) pepper plants to examine if the mycorrhizal interaction can increase host plant fitness.

Materials and Methods

Mycorrhizal and nonmycorrhizal peppers (Capsicum sp., California Wonder) were grown under controlled nutrient conditions in washed and sterilized mason's sand. Mycorrhizal peppers were inoculated with 2 g fresh onion roots infected by either Glomus mosseae, isolate A (code GM); G. mosseae, isolate B (code MO); or a combination of fungi extracted from an agricultural soil. The extracted fungi included G. mosseae, G. fasciculatum, and Gigaspora sp. (combination code MIX). Uninfected or nonmycorrhizal peppers (code NM) were inoculated with 2 g of fresh uninfected onion roots. Ten uninfected plants were grown at each of five progressively increasing phosphorus levels, while ten mycorrhizal plants were grown at the lowest (P-1) and the middle phosphorus levels (P-3). In addition, all the plants were fertilized equally with Long Ashton nutrient solution without hosphorus. At about the time the pepper fruits began to ripen (143 days in ths experiment), the roots, shoots and fruits of each plant were harvested, dried (80 C, until dry), and weighed. Reproductive effort was calculated for each plant as follows:

$$\text{REPRODUCTIVE EFFORT} = \frac{\text{dry wt. of fruits}}{\text{total plant dry weight}} \times 100$$

Roots were cleared, stained and examined for mycorrhizal infection (Phillips and Hayman, 1970). Samples of roots, shoots and fruits were analyzed for phosphorus levels using mixed-acid digestion and the molybdenum blue colorimetric procedure (Allen et al., 1974).

Results and Discussion

Pepper plants infected by mycorrhizal fungi were larger and generally produced a larger dry weight of fruits than uninfected plants. However, plants infected with MO and MIX were not significantly larger than the nonmycorrhizal controls (NM) at the P-1 and the P-3 phosphorus treatment levels. The reproductive effort of GM mycorrhizal plants was significantly greater than that of uninfected controls (NM) and the other mycorrhizal plants grown at the P-1 phosphorus level (see Table 1). At the middle phosphorus level (P-3), the mycorrhizal plants had greater reproductive effort than the nonmycorrhizal controls, but the differences were significant only for plants infected with MIX, the combination of mycorrhizal fungi. In all cases where the reproductive effort of mycorrhizal plants was greater than that of nonmycorrhizal controls grown at the same phosphorus level, the major change in the allocation of photosynthate in the mycorrhizal plants was a reduction in dry matter in the roots with an increase in dry matter committed to reproduction (see Table 1).

Table 1. Reproductive effort, allocation of photosynthate to plant structures, and total dry matter production (dry wt.) of mycorrhizal and nonmycorrhizal (NM) pepper plants at two phosphorus levels. Values in a row followed by the same letter are not significantly different (α=0.05). All values are means (n=10).

	INOCULUM TYPE			
	NM	GM	MO	MIX
LOW PHOSPHORUS LEVEL, P-1				
Reproductive Effort (% fruits)	18.8a	35.5b	18.5a	17.0a
% Shoots	47.5a	39.5b	50.6a	53.1a
% Roots	33.7a	25.0b	30.9a	29.9a
Total Dry Wt. (g)	2.32a	4.59b	2.83a	2.71a
MIDDLE PHOSPHORUS LEVEL, P-3				
Reproductive Effort (% fruits)	32.4a	---[1]	36.1a	40.3b
% Shoots	39.0a	---	40.6a	37.3a
% Roots	28.6a	---	23.3b	22.4b
Total Dry Wt. (g)	4.99a	---	5.76a	5.18a

1 Not available due to limited inoculum supply.

Reproductive effort was significantly correlated with total phosphorus accumulation (r=0.858) and with total dry weight (r=0.940). Although differences in responses were observed between fungal inocula and phosphorus levels, pepper plants generally had higher reproductive effort values when associated with the mutualist, VA mycorrhizal fungi, than when growing without the mutualists. Mycorrhizae increase host plant reproductive effort and fitness.

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THE OCCURRENCE OF VAM IN A SERPENTINE GRASSLAND COMMUNITY OF CENTRAL CALIFORNIA

By Natalie A. Hopkins

Keywords--Glomus tenue, Glomus fasciculatum, annuals, cover, soil core.

Introduction

VA mycorrhizae are common in grasslands according to studies reported from various parts of the world. In natural communities, mycorrhizae have been found on a majority of the plants. In one undisturbed community, 99% of the cover was found to be mycorrhizal.

In a grassland on shallow serpentine soil in the Coast Ranges of central California, mycorrhizae should be prevalent. This community differs from most previously studied communities because of the soil and because the plants are almost all annuals. For this reason a study was undertaken to determine the extent of mycorrhizae in the community as a whole, as well as in the dominant species.

Materials and Methods

The study site is at 396 m elevation on the eastern slope of the Santa Cruz Mts. The gravelly soil averages 6 cm in depth and has 7 ppm phosphorus. Common fungal symbionts in the soil are Glomus tenue, and Glomus fasciculatum.

Ten 10 m transects were used with ten randomly selected sampling points on each one. At each point a soil core was taken. The ten cores for each transect were combined. Subsequently, roots were washed free of soil, cut into one cm sections, cleared and stained. One hundred randomly selected sections were mounted on slides and used to determine the percent colonization by length for roots in each transect.

Equidistant on either side of the transect from those same points, vegetation was sampled by using a double-sighting cross method to make a point-intercept (Mueller-Dombois and Ellenberg 1974). Each transect provided 20 samples. These were used in the determination of cover.

Ten of each annual species contributing to cover were collected and the roots cleared, stained and examined microscopically. Classification of the percentage of feeder roots colonized and also the intensity of the colonization was made for each species using Kormanic and McGraw's (1982) Non-Systematic Method. Five classes were used for colonization ranging from one (no colonization to 5%) to five (75% to 100%). Three classes were used for intensity ranging from one (only scattered colonization sites) to three (almost solidly colonized).

Results and Discussion

The range of colonization in root sections from cores was similar for each transect. The means ranged from 91% to 99%. Of all sections examined, 85.8% of them had 100% colonization and 97.3% had more than 50% of each section colonized. Only 1.2% of the sections had 10% or less colonization; 0.5% of the sections were without hyphae. Vesicles and arbuscules were common. It is likely that the 2 cm soil corer picked up only feeder roots because the average diameter of these roots was 0.22 mm.

Herbaceous plants made up 96% of the cover. Ninety percent of the cover was identified to species which were collected and classified. All but four of the 27 species were annuals. Annuals made up 80% of the cover. All annual forbs, except two species from nonmycorrhizal families, had the highest classification for both colonization (class 5) and intensity (class 3).

The amount of herbaceous cover occupied by species with root systems that were more than 50% colonized was 97.8%. This corresponds to the 97.3% found in roots from the soil cores.

The amount of herbaceous cover occupied by species with root systems with 5% or less colonization was 1.7%. This is slightly more than the 1.2% from the root core sections with 10% or less colonization. But one can safely conclude that this community, although populated mostly by annuals, has root systems composed largely of VA mycorrhizae.

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VA SPORES IN VA SPORES

By

R.E. Koske

Keywords--Gigaspora, Acaulospora, Glomus, sand dunes

Introduction

In a survey of VAM fungi associated with sand dunes colonizing plants of the Atlantic Coast of the U.S. and of the Great Lakes, numerous dead spores of VA fungi were found to be occupied by spores of other VA fungi. Because the presence of spores within spores may be of ecological significance and also may lead to taxonomic confusion, the following study was initiated to catalog the species involved.

Methods and Materials

Soil samples were collected from root zones of the dominant plant species on maritime dunes from Nova Scotia to Virginia, and from lacustrine dunes on the eastern shores of Lake Michigan and Lake Huron. Spores were extracted from soil by wet-sieving and filtration.

Results and Discussion

Spores of 11 species of VA fungi were occupied by spores of other VA species (Table 1). The most frequent occupant was a species of Glomus similar to one described by Rosendahl (1943) as the fossil species Rhizophagites butleri (Fig. 1).

Table 1. VA spores occupying VA spores

Species whose spores were occupied	Species whose spores were occupants
Acaulospora scrobiculata	A. scrobiculata
Gigaspora calospora	A. spinosa
Gi. dipapillosa ined.	Gi. calospora
Gi. erythropa	Gi. erythropa
Gi. fulgida ined.	Gi. fulgida
Gi. gigantea	Gi. gigantea
Gi. pellucida	Gi. pellucida
Gi. persica ined.	Gi. persica
Gi. verrucosa ined.	Gi. reticulata
Gi. sp.	Gi. sp.
Glomus tortuosum	Gl. tortuosum
	Gl. sp.

Three types of occupancy were observed:

a) single species occupancy-
   1-100 spores of a single VA species occupied a spore.
   Example: Glomus inside Gigaspora

b) multiple species occupancy-
   spores of 2-5 different VA species occupied the same single spore.
   Example: Glomus and Acaulospora inside Gigaspora

c) nested occupancy-
   spores inside spores were themselves occupied by other VA spores.
   Example: Glomus inside Acaulospora inside Gigaspora inside Gigaspora

Dead VA spores appear to provide favorable microhabitats for sporulation by other VA fungi, much as do dead seeds and seed coats, pieces of insects and other organic matter in the soil. Although there was no direct evidence that one VA species could invade healthy spores of another species, the ratio of occupied dead spores to healthy spores in some samples was 5:1 and suggests further investigation.

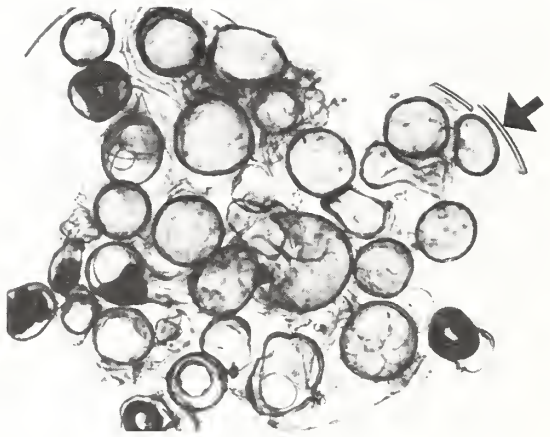

Figure 1. Crushed spore of Gigaspora gigantea occupied by spores of Glomus sp. Arrow indicates Gigaspora wall. X175.

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VAM IN EQUISETUM

By

R.E. Koske, C.F. Friese, R.L. Hauke, and
P.D. Olexia

Keywords--sand dunes, Michigan

Introduction

The mycorrhizal status of species of Equisetum has been the subject of controversy in recent years. Typical VAM were noted in 2 species of Equisetum in Rhode Island in 1981 (2), confirming earlier reports on the VAM status of the group. Berch & Kendrick (1) examined 30 root systems of several species of Equisetum from Ontario, Canada, and found levels of VAM to be minimal or zero. Based upon their results and their interpretation of the work of previous investigators, they suggested that the demise of the Equisetales was a result of the inability of its members to form VAM and, thus, successfully compete with mycotrophic higher plants.

Because non-host plants may be colonized to a limited extent by VA fungi when non-host and host plants grow in close proximity (3), we believe that it was necessary to re-investigate the VAM status of Equisetum to resolve the question of whether the genus is mycotrophic or not.

Methods and Materials

Sporophyte plants of 3 species of Equisetum were collected from sand dunes at the Grand Sere area of Lake Michigan in July & Sept., 1983. Care was taken to collect plants that were not growing in close association with other dune-inhabiting species. Roots were cleared in KOH, decolorized in hypochlorite, stained with trypan blue, and examined at 100-400 X. Intensity of VAM development was assessed by estimating the percent of root length containing hyphae, arbuscules or vesicles.

Results and Discussion

Seventeen of the 19 plants examined bore VAM. Levels of colonization varied from 0 to 90%.

Table 1. Percent colonization of roots of Equisetum

Equisetum species	% of root length colonized*
E. arvense	10
E. hyemale v. affine	0,0,10,10,10,10,10, 10,20,30,50,50,50, 70,70,80,90
E. x ferrissii	10

*of individual plants

The presence of arbuscules in the root systems and/or the high levels of colonization indicated that these 3 species of Equisetum are capable of functioning as host-species in VAM relationships. As noted in previous studies, Equisetum appears not to be an obligately mycotrophic genus, but may develop VAM under certain conditions. The extremely low nutrient status of the sand dunes may have encouraged formation of VAM. This could explain the difference between the results of this study and those of Berch and Kendrick who sampled from mixed woodland soils.

We suggest that the demise of the Equisetales was more likely a result of the worldwide change from hydric to mesic conditions in the past, rather than from the ability or inability of this group to form VA mycorrhizae.

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SELECTIVE INFLUENCE OF VAM ON SOME FUNCTIONAL GROUPS OF RHIZOSPHERE BACTERIA AND ACTINOMYCETES

Julie Meyer and Robert G. Linderman

Introduction

The effect of VAM on root growth, morphology and metabolism may result in secondary interactions with other microorganisms intimately associated with roots.

The purpose of this study was to examine the possible qualitative influence of the VAM symbiosis on microorganisms naturally found on roots. General taxonomic and functional groups of bacteria and actinomycetes were assayed in both the rhizosphere and rhizoplane.

Materials and Methods

Nonsterile soil free from indigenous VAM fungi was prepared by inoculating pasteurized sand with a garden soil suspension passed through a 38 μm sieve. Sweet corn was inoculated at sowing with root pieces colonized by Glomus fasciculatum, controls (NMR) received nonmycorrhizal root pieces. In subsequent experiments with subterranean clover, harvested at 6 and 12 weeks, VAM spores rather than roots were used to minimize microbial contamination.

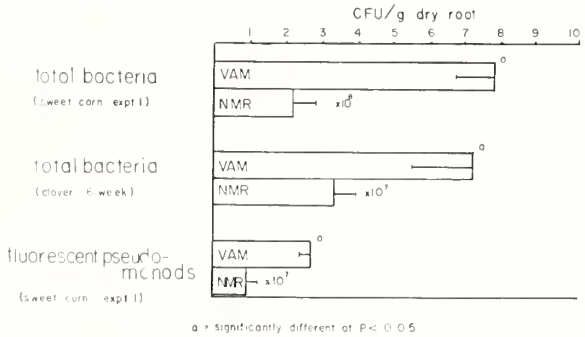
The rhizosphere soil sample was obtained by gently shaking roots with adhering soil in a 0.1% water agar dilutent. The rhizoplane sample was obtained by macerating the washed roots in a sterile blender.

Fluorescent Pseudomonas spp., chitinase-producing actinomycetes, Streptomyces spp., facultative anaerobic bacteria, gram-negative bacteria, total bacteria and total actinomycetes were assayed on selective media. Microbial activity influencing behavior of Phytophthora cinnamomi was assayed by the sporangia production method on agar plugs in VAM and NMR clover rhizosphere soil extracts. At least five plants were harvested per treatment in all experiments.

Results and Discussion

Establishment of VAM increased total bacterial populations but did not affect numbers of actinomycetes on the rhizoplane (Fig. 1). Neither group was affected in the rhizosphere soil.

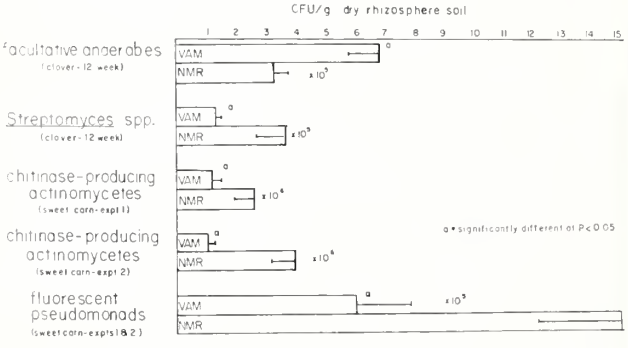
Fig 1 Populations of bacteria on the rhizoplane of VAM and nonmycorrhizal (NMR) roots



a = significantly different at P< 0.05

However, VAM affected specific groups of bacteria and actinomycetes in both the rhizosphere and rhizoplane (Fig. 1 and 2). VAM increased numbers of facultative anaerobic bacteria (possible N_2-fixing) isolated on low N medium, decreased numbers of total fluorescent Pseudomonas spp. but had no effect on total numbers of gram-negative bacteria. Of the actinomycetes assayed, populations of both Streptomyces spp. and chitinase-producing actinomycetes decreased in the VAM rhizosphere (Fig. 2).

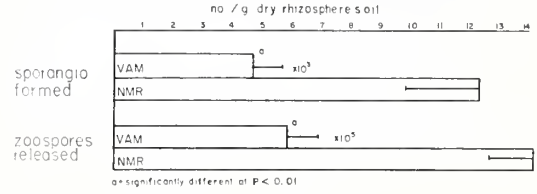
Fig 2 Populations of bacteria and actinomycetes in the rhizosphere of VAM and nonmycorrhizal (NMR) roots



a = significantly different at P< 0.05

Leachates of VAM rhizosphere soil suppressed P. cinnamomi sporangia production compared to leachates of NMR rhizosphere soil (Fig. 3). These results suggest that sporangia-inducing microorganisms had declined or sporangia-inhibitors had increased. The number of Rhizobium nodules/mg dry root increased significantly from .13/mg dry root to .73/mg dry root on 6 wk old VAM clover.

Fig 3 Sporangia and zoospore production by Phytophthora cinnamomi in VAM and nonmycorrhizal (NMR) root rhizosphere soil extracts



a = significantly different at P< 0.01

Although VAM significantly enhanced growth in the 6 wk old clover experiment, P and N concentrations did not differ between VAM and NMR roots and shoots. Changes in root exudation patterns in VAM plants probably best explain the results of this study, but one should also consider that the VA-mycorrhizal fungus itself may exert a selective influence over root microflora by means of spore or hyphal exudation.

Conclusion

The establishment of VAM has a selective influence on populations of specific groups of bacteria and actinomycetes associated with roots.

Shifts in populations of functional groups of microorganisms due to VAM establishment may have some important effects on plant growth: microorganisms produce and metabolize growth-promoting substances, affect nutrient availability, rhizosphere pH and root pathogens. The net effects of these microbial activities in relation to VAM is an unexplored area of research which may lead to new interpretations of the effect of mycorrhizal fungi on plants.

THE EFFECTS OF TIME AND ASPECT ON SOIL VAM INOCULUM AFTER DISTURBANCE

By

M. E. Waaland and E. B. Allen

Keywords--succession, reclamation, inoculum reestablisment, Agropyron smithii, Salsola kali, Kochia scoparia, stripmine

Introduction

Primary succession occurs on mineral substrates such as stripmine spoils. VAM spore density is low on these sites and may remain so indefinately should non-mycotrophic annuals persist. Wind is the primary vector of dispersion in arid regions and spores are deposited differentially on east and west aspects (Warner, 1984). VAM inoculum may act to regulate the establishment of late seral mycotrophs (Allen, 1984) suggesting succession can be accelerated where adequate inoculum is present.

A field survey was conducted in 1983 on different aged reclaimed coal stripmime sites to assess the effects of time and aspect on the reestablishment of VAM inoculum and vegetation.

METHODS

The stripmine is in SW Wyoming and receives 23 cm precipitation annually. The 3 study sites chosen were revegetated with a standard seed mix on spoil in 1978, 1980, and 1981. Pioneer species were predominately non-mycotrophic annuals of the Chenopodiaceae, such as Salsola kali and Kochia scoparia. Polygonum arvense was common also.

Twenty m transects were established on windward (west) and lee (east) slopes parallel to the ridgeline no less than 5 m from the crest. Cover estimates were made to the nearest 1% with a 0.5 m^2 plot frame at randomly chosen points along the transect. One root sample and two composite soil samples were taken for each of the 5 transects run per site. Roots were washed, stained, and per cent infected intercepts counted. Spores were separated using sucrose flotation and counted. Data were subjected to one-way ANOVA. Data were not available for the east aspect of the 1978 site.

Results and Discussion

Roots of A. smithii were infected within two years of revegetation, although percentages were low on all sites (Table 1). Only for the east aspect of the 3 year old site did spore density differ from the rest (Table 1). Spatial variability may be too great for significance at this scale of sampling.

Cover increases with time for perennials and decreases for annuals (Table 2). For east aspects, perennial cover is greater but annual cover decreased by year 3 suggesting an acceleration of succession on these sites.

Finally, spore density is not strongly correlated with perennial grass cover (Fig.1).

Table 1. Soil spore density and root infection of A. smithii.[1]/

Yr. since reveg./ aspect		%infection A. smithii	# spores/gr dry soil
2	W	6.7a	5.8a
	E	4.8a	5.0a
3	W	8.0a	10.1a
	E	18.0a	19.7b
5	W	15.2a	10.8a

Table 2. Mycotrophic perennial and non-mycotrophic annual cover.[1]/

Yrs. since reveg./ aspect		% Cover A. smithii	A. dasystachum	Annuals
2	W	1.4a	1.4a	20.7a
	E	0.3a	4.4b	31.1b
3	W	6.1b	11.0c	3.7c
	E	14.0c	17.4d	0.4d
5	W	10.3d	22.0e	0.2d

[1]/Those means within a column not sharing a common letter differ significantly. L.S.D. 0.05.

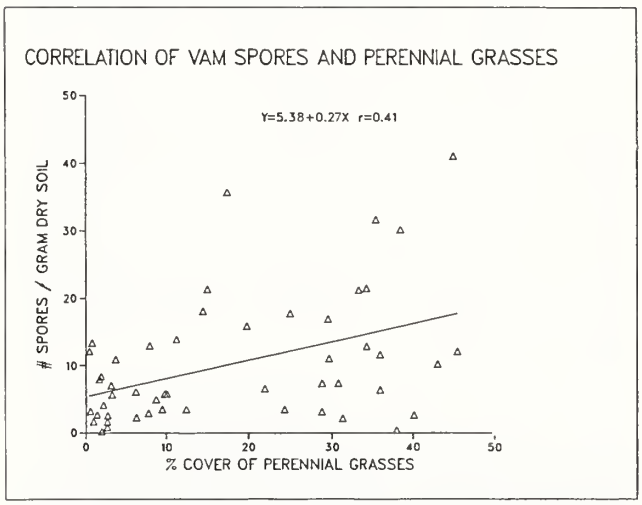


Figure 1. Relationship between spore density and perennial grass cover. Coefficients are significant (alpha=0.05).

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STABILITY OF CHLAMYDOSPORE AND MYCORRHIZA MORPHOLOGY IN DISSIMILAR PLANT AND SOIL ENVIRONMENTS.

Joseph B. Morton, Div. of Plant and Soil Sciences, West Virginia Univ., Morgantown, WV 26506

Key words: Glomus occultum, Glomus diaphanum, taxonomy.

Introduction

Taxonomic criteria differentiating VA mycorrhizal fungi in the Endogonaceae are based solely on spore or sporocarp morphology (3). Validity of these parameters rests on the assumption of phenotypic stability across diverse environments. In this study, the range of variation in chlamydospore and mycorrhiza morphology of two hyaline-spored species, Glomus occultum and Glomus diaphanum, was examined on different plant hosts growing in selected soils with dissimilar physical and/or chemical properties.

Materials and Methods

Root-soil mixtures were collected from the rhizosphere of corn, fescue, red fescue, black locust, red clover, and broomsedge at seven field locations. All soils were physically and chemically characterized. Glomus occultum or G. diaphanum spores were extracted from triplicate soil samples collected at each site and examined under a light microscope to compare spore size, shape, color, and structure.

Spores of each isolate were pooled from triplicate soil samples at each location and inoculated onto corn, fescue, black locust or red clover in pot cultures (sterile soil:sand, 2:1 v/v). Spore and mycorrhiza morphology were examined in material collected from pot culture hosts grown for 4 months.

Corn, fescue, sudangrass, and red clover were inoculated with G. occultum and G. diaphanum in 100-ml cone-tainers. Endophyte colonization was followed during mycorrhizae development over an 80-day growth period.

Results and discussion

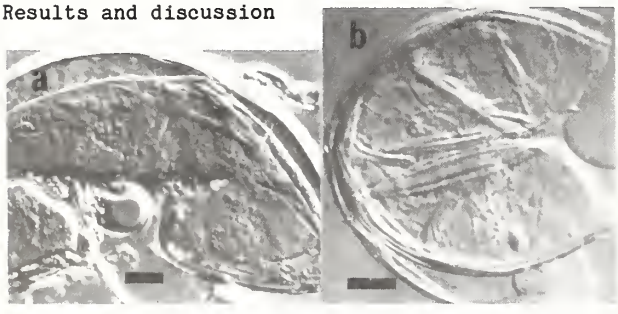

Figure 1. Crushed spores of a) G. diaphanum and b) G. occultum. Bar represents 10 µm.

Spore wall structure readily separated G. occultum and G. diaphanum. Variation was expressed among isolates of G. occultum as the extent to which the two thin inner walls were adpressed and among G. diaphanum isolates as the extent to which laminae of the thick outer wall separated into discrete layers. Intraspecific variation of other characters (including spore size, shape, and color) was not significant across the range of plant species tested or across soils with a wide range in physical and chemical properties. Intraspecific variation did not obscure differences in spore diameter between species, even with broad overlap. Spores of both species were hyaline throughout their life cycles, regardless of plant or soil environment.

Mycorrhiza morphology also differentiated the two endophyte species. Intraradical arbuscules and hyphae became indistinct within two weeks after root penetration by G. occultum. Similar mycorrhizae development is shared by G. fecundisporum, G. leptotichum, and G. tortuosum (2). None of the hosts in the time course experiment showed any infection at 80 days, even though extraradical spores reached levels of 178 to 237 spores ml^{-1} in soil planted to fescue and sudangrass, respectively. Glomus diaphanum produced typical arbuscules and vesicles, but also formed abundant intraradical spores at the expense of vesicle development (see Figure 2). Although mycorrhiza ontogeny was consistent across all hosts, the rate and degree of infection varied with plant species.

Lack of significant environmental impact on spore morphology suggests that current taxonomic criteria reliable separate VA mycorrhizal fungi. Mycorrhiza morphology, which is more significant biologically, differed sufficiently in this study and elsewhere (1, 2) to warrant greater consideration in classification.

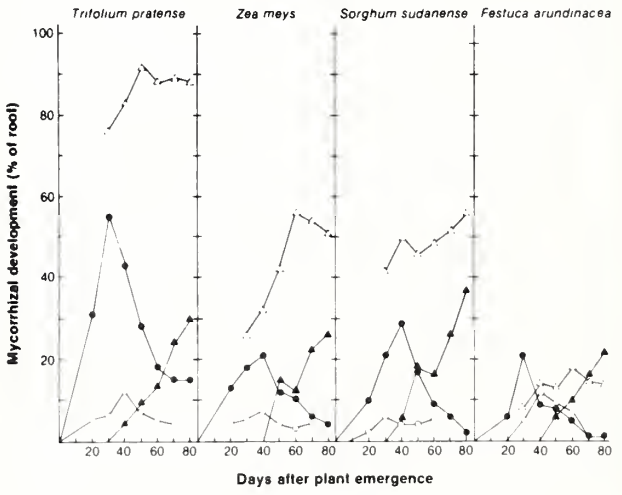

FIGURE 1

Mycorrhizal development of Glomus diaphanum in four host species. -●- arbusoule, -○- vesicle, -▲- spore formation in the root cortex as a function of the mean percentage colonization of 50 one-cm mycorrhizal segments, and -△- mycorrhizal incidence in the whole root system.

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VARIATION IN MPN'S WITH INTERACTIONS BETWEEN NATIVE VAM FUNGI, HOSTS, AND SOILS. Mitchell Adelman and Joseph Morton, Div. of Plant and Soil Sciences, West Virginia Univ., Morgantown, WV 26506.

Key words: Root density, inoculum potential, infectivity, adaptation.

Introduction

The Most Probable Number Method has been used to enumerate total infectious propagules of soil-borne VA mycorrhizal fungi (2). Experimental conditions strongly influence MPN estimates (3). In this study, infectivity of native VA mycorrhizal fungi in soils with dissimilar properties was estimated using different plant hosts in MPN tests.

Materials and Methods

Corn, fescue, black locust and apple were grown in 50-ml cone-tainers filled with sand for variable lengths of time and at different seeding rates. A root-soil-spore inoculum mixture of Glomus diaphanum was mixed with the sand to monitor mycorrhizal incidence. Root volumes were measured at each harvest by displacement in water. All MPN tests were performed using the seeding rate and growth period of each host at which root densities were approximately equivalent and mycorrhizal infection had stabilized. (see legend, Figure 1).

Root/soil mixtures were collected from the rhizospheres of corn, fescue, black locust, and apple growing continuously at selected sites for over 9 years. A portion of each soil was autoclaved twice and re-inoculated with native microflora (<8 um) for use as diluent. Each MPN test consisted of 2-fold dilutions with 5 replications. Infectivity of native VAM fungi in each soil was estimated across all four soil environments (as sterilized diluent) with the host being the predominant plant species at the inoculum source location (see Figure 1).

Results and discussion

Comparison of mycorrhizal infectivity in selected soil environments using different plant species required that root densities be equivalent. Consequently, variation in propagule-root contact was reduced.

Indigenous endophytes are known to be more efficient symbionts in soils to which they are well adapted (1). In this study, indigenous VA mycorrhizal fungi were most infective in the soil-host environment which best approximated native conditions (Figure 1). In addition, more abundant root colonization, sporulation, and extramatrical hyphae production were observed in initial dilution tubes (1/64) of the best adapted inoculum-diluent-host combinations. For example, Glomus intraradices comprised 88% of the total spore count in the fescue inoculum. Infection in fescue roots from the sample site and from the fescue inoculum-fescue soil diluent combination in the MPN was 65-72%. However, infection of fescue roots by G. intraradices from another soil source added to the sterilized fescue soil never exceeded 18%.

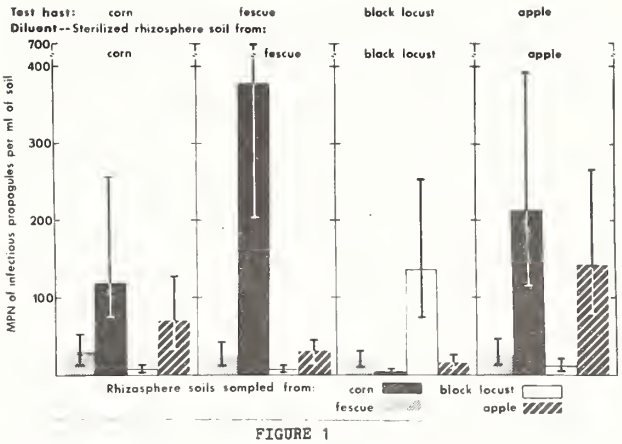

FIGURE 1

Comparison of the MPN of infectious VA endophyte propagules in rhizosphere soils using different host-diluent combinations. Seeding rate/growth period (weeks) for each host was: Corn (1/5), fescue (8/6), black locust (2/10) and apple (2/10). Fiducial limits (95%) are indicated by vertical bars.

The black locust soil was very high in Al (91% of total cations), yet infection by native endophytes was not deterred. Soil-borne spores and extramatrical hyphae were observed only in the black locust inoculum together with black locust as host and the same soil as diluent. In addition, infection was 3-fold higher than in other soil diluents. Apple showed similar host-endophyte-soil diluent interactions when native edaphic conditions were matched. Similar infectivity of corn inoculum across diluent environments may indicate the absence of selective adaptation.

The MPN method estimated inoculum potential rather than inoculum density, as evidenced by disparity between spore counts and infectious propagules. Not only propagule density, but physiology of the host, root susceptibility to infection, soil environmental conditions, and interactions among inoculum components determine inoculum potential. The fescue inoculum-diluent-host combination was much higher (374 propagules ml^{-1}) than either fescue seeded to the same inoculum diluted with sterile sand (27 propagules ml^{-1}) or spore density (78 spores ml^{-1} extracted from a root-soil mixture blended before sieving). Sand, a relatively inert medium, consistently underestimated inoculum potential. Thus, MPN estimates of native endophytes in a field soil appear to be optimized only when the diluting medium approximates edaphic conditions of the inoculum.

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EFFECT OF CULTIVAR AND LOCATION ON INFECTION OF ARACHIS HYPOGAEA L. BY INDIGENOUS VAM FUNGI IN TEXAS

By

J. S. Neck, R. A. Taber, T. D. Riley, R. E. Pettit, O. D. Smith, R. M. Taylor, K. E. Woodard, D. Smith, and T. Boswell

Keywords--Glomus deserticola, Sclerocystis sinuosa, saline, phosphorus, groundnut

Introduction

Peanuts are an important oil and protein source grown on a world-wide basis over 18,000,000 ha. In the U.S., 566,572 ha. were planted in 1983, but only 554,431 ha. were harvested, mainly due to drought loss. Texas is one of seven states that account for 98% of peanut production in the U.S. Both in Texas and on a world-wide basis, the availability of good quality water and suitable croplands continue to be a growing problem.

Vesicular-arbuscular mycorrhizal fungi have been shown to increase peanut growth in greenhouse studies. A pilot study was initiated in Texas to assess cultivar differences in regard to indigenous VAMF acceptance. Sites chosen included: a commercial production area (Stephenville), two peanut breeding experimental plots (Bryan and Yoakum) and an adverse site never planted to peanuts (El Paso).

Materials and Methods

Ten peanut cultivars were selected to represent a broad range of genetic background: Virginia types (bunch and runner), Spanish, and Valencia. Four replications of each cultivar were planted at each of four sites in Texas. Cultural practices used were those recommended for commercial peanut production. The adverse site at El Paso was irrigated with saline water (1.25 dS/m).

Whole plants were collected and the fine feeder roots were sampled at various times during the growing season. Only data at 1 1/2 months are presented here. Root samples were cleared, stained and mounted in lactophenol blue. A nonsystematic class ranking on a 1 to 5 scale was used to facilitate quick assessment of mycorrhizal colonization. Composite soil samples from each test location were wet sieved and separated by density gradient centrifugation.

Results and Discussion

Extent of colonization by indigenous VAMF varied among peanut cultivars and locations as indicated by the preliminary data (Table 1). Roots from the Stephenville test plots contained the highest concentration of mycorrhizal development in a soil low in phosphorus (14 ppm). In contrast, roots from the El Paso plots had the lowest degree of colonization overall, in soil relatively high in phosphorus (>84 ppm) and salinity. Exceptions to the general decrease in colonization at El Paso included cultivars NC8C, TP 107-27-1y, and Florunner. Mycorrhizal fungus development was least affected by the adverse conditions in cultivar NC8C, a Cylindrocladium resistant Virginia bunch type.

The average ranking of mycorrhizal colonization from all four sites was highest on cultivar Florunner and PI 365553, both runner types. In other comparative yield tests, these two cultivars frequently have higher yields compared to the other cultivars. Peanut plant survival was not related to the degree of mycorrhizal colonization. Aspergillus niger crown rot was responsible for stand losses throughout the test plots early in the season.

Extensive chlorosis exhibited by several cultivars at El Paso was attributed to the lack of available iron and/or Rhizobium nodules. Species in the genera Glomus and Sclerocystis occurred most frequently, with Gigaspora sp. infrequently occurring. No Gigaspora species was observed in the El Paso soil; however sporocarps of Sclerocystis sinuosa were common. Pot cultures of the various mycorrhizal isolates are currently being established.

TABLE 1. MEAN CLASS RANKING OF PERCENT OF FINE ROOTS INFECTED APPROXIMATELY 1½ MONTHS AFTER PLANTING

		EL PASO	STEPHENVILLE	YOAKUM	BRYAN
PEANUT CULTIVAR	BOTANICAL TYPE [C]	RANKING [AB]			
NC8C	VA. BUNCH	4	3	3	2
TP 107-27-1Y	VA. RUNNER	3	2	2	3
FLORUNNER	VA. RUNNER	3	5	3	4
PI 337409	VALENCIA	3	5	5	1
PI 365553	VA. RUNNER	2	5	5	4
TOALSON	SPANISH	2	•	3	2
PI 296551	VA. RUNNER	2	5	3	2
STARR	SPANISH	1	5	3	3
PI 300596	VA. RUNNER	1	2	4	•
TAMNUT 74	SPANISH	•	•	3	2

[A] VARIABLE-NO RELIABLE DATA AVAILABLE

[B] 1 = 1-20%, 2 = 21-40%, 3 = 41-60%, 4 = 61-80%, 5 = 81-100%

[C] ARACHIS HYPOGAEA L.
SUBSPECIES HYPOGAEA
VAR. HYPOGAEA - VIRGINIA
VAR. HIRSUTA - CHINESE
SUBSPECIES FASTIGIATA
VAR. FASTIGIATA - VALENCIA
VAR. VULGARIS - SPANISH

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THE EFFECT OF ENDOMYCORRHIZAE ON INTERACTIONS BETWEEN MYCORRHIZAL AND NONMYCORRHIZAL PLANTS

BY: Raymond Franson and R. Michael Miller

Keywords:--Atriplex, Sitanion, Glomus, interaction, competition, host specificity

Introduction

Plant ecologists interested in competition have typically observed plant-to-plant interactions, measuring plant responses without reference to potential microbial interactions. Mycorrhiza research in the past has been focused around host and endophyte interactions with very few studies to date on interspecific plant responses. Thus, an investigation was initiated on how infection with vesicular-arbuscular mycorrhizal fungi (VAM) changes interactions between plants. Specifically addressed were how does the presence or absence of VAM (Glomus monosporum/mosseae) affect: 1) a species that typically shows little or no infection (Atriplex canescens), and 2) a species that typically possesses moderate levels of infection (Sitanion hystrix).

The three species used in this study occur together in the Red Desert of Wyoming.

The three species interaction (Sitanion, Atriplex, Glomus) on growth parameters was studied by comparison of two de Wit replacement series. One series is inoculated with Glomus, the second series is uninoculated.

Procedures

Soil collected from the Red Desert in Wyoming was diluted with sand (4:1 v/v), fumigated with methyl bromide, and aerated. Spores of G. monosporum/mosseae obtained from soil collected at the Red Desert site were seived and mixed into the treated growth medium as a wash at a rate of 0.15 g^{-1} soil. A spore-free wash (<38 µm) was added to the uninoculated treatment. Seedlings of S. hystrix and A. canescens were planted at a density of 3 plants/container in all four possible combinations necessary for a de Wit replacement series (3:0, 2:1, 1:2, 0:3, Sitanion to Atriplex). The containers were 1 liter cups lined with plastic bags and contained 1000 g of soil. Soil was watered to field capacity (12%) twice/wk throughout the experiment. Plants were grown in a growth chamber at 10°C, 16°C, and 20°C for 31, 31, and 28 days (12 hr L/D, 550 µE/m^2s, 40% relative humidity), respectively, approximating the seasonal temperature fluctuation at the Red Desert site. Each replacement series was replicated six times giving a total of 48 containers (4 x 2 x 6) and 144 plants. Roots were separated by species at harvest, and below-ground plant weight and fungus % root length colonized was determined.

Results and Discussion

Very few points of entry of mycelium or internal structures could be found in either Sitanion or Atriplex roots. All root samples were less than 1% infected by the grid-line intersect method. However, mycelium of Glomus was often very abundant around inoculated Sitanion roots, sometimes wrapped tightly around the roots. Inoculated Atriplex plants also contained Glomus mycelium around the vicinity of their roots, though not at the levels found for Sitanion plants.

The root dry weights for inoculated Sitanion plants were higher than the uninoculated treatments (Fig. 1). The significance of the difference between the two curves is obscured by a container effect where the 3:0 Sitanion to Atriplex inoculated treatments were limited by pot size explaining the leveling off of this curve. The aboveground dry weight gain for the 3:0 Atriplex to Sitanion treatments were significantly higher with inoculation (Fig. 2). The difference between inoculated and uninoculated Atriplexes was not significant for aboveground biomass when Sitanion was present in the pot. The growth responses of both S. hystrix and A. canescens suggest a fungus-to-plant interaction even though infection, as measured by arbuscules, or vesicles, was not always present. The disappearance of the Atriplex-Glomus interaction in the presence of Sitanion suggests an important Sitanion-Atriplex interaction.

The lack of high infection levels of G. monosporum with either S. hystrix or A. canescens suggest possible host specificity. The soil P levels in the Jim Bridger site vary from 2 ppm to 30 ppm. The soil used in this experiment had P levels of 20 ppm. This is relatively high and may also explain the low infection levels.

Figure 1. de Wit Replacement Series For Atriplex versus Sitanion

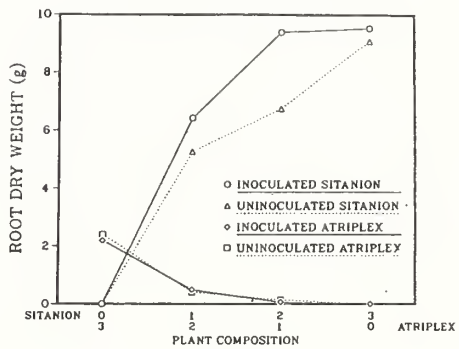
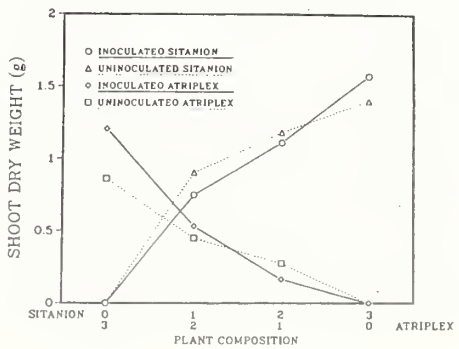


Figure 2. de Wit Replacement Series for Atriplex versus Sitanion



MYCORRHIZAE: EFFECT ON PLANT PRODUCTIVITY IN GREAT BASIN COAL-MINE SOILS

by

C. Coe Klopatek, J.M. Klopatek

Keywords-- Atriplex, Glomus, reclamation

INTRODUCTION

Stripmining for coal leads to physical and chemical changes in the soil which in turn affect the biological organisms. Recently, coal mine reclamation studies have demonstrated the need for the reestablishment of the below ground symbionts (e. g., mycorrhizae) necessary for a stable plant community, as many plant species can not survive without these symbionts. The objectives of this study were to: 1) examine the effects of mining and reclamation activites on mycorrhizal populations and in turn how this affects the restoration of the dominant shrub species; 2) evaluate the soils of these disturbed and undisturbed systems to determine if they can support mycorrhizal associations; and 3) determine the influence mycorrhizae have on the growth of some of the native species used for reclamation.

This study was conducted at Black Mesa, located on the Navajo and Hopi Indian reservations, in northeastern Arizona. This area is currently undergoing stripmining for coal in excess of 150 ha/yr. The region is dominated by a pinyon-juniper woodland community with a mosaic of gramma-galleta steppe and sagebrush communites. Reclamation efforts consist of topsoiling, mulching with straw and seeding with non-native grass species interspersed with Atriplex canescens. Soils were analyzed from four undisturbed plant communities Pinus edulis-Juniperus osteosperma, Artemesia tridentata, Gutierrezia sarothrae-Chrysothamnus spp., Atriplex canescens; three reclaimed soils, one prelaw-without topsoil covering and two postlaw - with topsoil covering, one having an extra layer of

MATERIALS AMD METHODS

In the first experiment, sudan grass was planted in sterile and unsterile soils to reveal the infection potential from each soil site. Sudan grass was grown under greenhouse conditions for a period of 49 days and 124 days. Upon harvesting, the roots were stained than scored for the presence or absence of mycorrhizae. A positive indentification consisted of the presence of hyphae, vesicles, arbuscules or any combination of the three. Growth measurements of roots and shoots were obtained at harvesting. In the second experiment Atriplex canescens was grown in sterile, unsterile, inoculated and noninoculated soils from each soil site. Germinated seeds were planted and grown for 95 days. Spores of Glomus mosseae or G. fasciculatus fungi were used as the fungal inocula in both sterile and unsterile soils. Mycorrhizal assessment and growth measurements were obtained as described in the first experiment.

Results and Discussion

Sudan grass infection percentages ranged from 45 to 78 in the natural soils to 10 to 52 in the reclaimed soils after 49 days; after 124 days, infection percentages decreased in both systems. However, infection percentages of sudan grass grown in topsoil showed an increase with time ranging from 5 to 50% at 49 d and 20 to 72% at 124 d. The data revealed that as the topsoil increased with age, electrical conductivity increased and infection percentage decreased significantly ($r^2 = 0.90$, $p < 0.05$). Plants grown in natural soil had a greater biomass compared to plants grown in reclaimed soils at both sampling times. The results of the second experiment with Atriplex revealed a positive correlation between percent infection and total biomass, with the exception of plants grown in unsterile inoculated topsoil storage piles. This contrasts to the dramatic increase in inoculated topsoil storage piles. Atriplex grown in unsterile and sterile soils inoculated with G. mosseae had a higher biomass production in the natural communities than in the reclaimed. Plants grown in sterile inoculated soils showed a significant increase in biomass and percent infection compared to the unsterile. Atriplex grown in stored topsoil showed the most dramatic effect when inoculated. Experiments repeated with G. fasciculatus exhibited similiar growth results. The data showed for both types of inocula there is a higher percent infection in the natural communities compared to the reclaimed. There was a positive correlation between total biomass and percent infection ($r^2 = +.76$, $p<0.01$). There also exists a statistically significant difference in percent infection between all three soils types. Sterile and unsterile noninoculated natural soils yielded a higher total biomass than the reclaimed. This study demonstrated: 1) the detrimental effects of disturbance on native mycorrhizal populations; 2) mycorrhizal infection rates decreased and soil electrical conductivity increased according to the length the topsoil storage pile was stored; 3) with the drastic disturbance of soil, mycorrhizae alone, in some instances, can not improve plant production.

Growth of Atriplex canescens in unsterile inoculated soils with Glomus mosseae

mg dry wt/plant	NATURAL				RECLAIMED			TOPSOIL		
	RABT	SAGE	P-J	SALT	PRE	EXTD	POST	1 YR	2 YR	3 YR
shoots	91	10	11	5	N/A*	4	2	8	9	11
roots	93	68	66	21	N/A	10	3	13	28	38
total	133	78	77	26	N/A	14	5	21	37	49
r/s	2.76	6.80	6.00	4.20	N/A	2.50	1.50	1.62	3.11	3.45

*N/A = ALL SEEDLINGS FAILED TO PROVIDE APPRECIABLE AMOUNTH OF GROWTH

Growth of Atriplex canescens in sterile inoculated soils with Glomus mosseae

mg dry wt/plant	NATURAL				RECLAIMED			TOPSOIL		
	RABT	SAGE	P-J	SALT	PRE	EXTD	POST	1 YR	2 YR	3 YR
shoots	91	36	78	27	14	59	5	113	65	32
roots	118	42	162	25	19	51	7	186	110	78
total	209	78	240	52	33	110	12	299	175	110
r/s	1.30	1.16	2.07	0.97	1.35	0.86	1.40	1.64	1.69	2.43

INTERACTIONS OF ARTEMISIA TRIDENTATA AND THREE SPECIES OF GLOMUS

By

Peter D. Stahl and S. E. Williams

Keywords--Artemisia tridentata, Glomus, effectivity

Introduction

As part of a program to produce mycorrhizal native shrubs for use in revegetation projects in Wyoming, various pot cultured VA fungi are being screened to find effective endophytes for regular use in greenhouse shrub production. Glomus species are the most commonly observed VA fungi associated with Artemisia tridentata (Big sagebrush) in Wyoming and many isolates of endophytes from this genus are available in pot culture form. A suitable symbiont should have the ability to infect and establish well on the root system of sagebrush and increase survival and vigor in the field. In this study, the intensity and anatomy of VAM formed by Glomus fasciculatum, G. macrocarpum, G. mosseae and native endophytes will be compared (Abbott, 1982).

Materials and Methods

Seed of A. tridentata was planted in pot culture soil of each of the three Glomus species, native sagebrush-grassland soil and pasteurized pot culture soil. Plants were harvested 30 days after seedling emergence. After immersion and soaking in warm water for 10 minutes, roots were carefully removed intact from soil and gently washed. Initial observation of roots with a binocular dissecting scope allowed examination of gross root morphology and external VA hyphae. The root system was then cleared in warm KOH and stained in lactophenol-trypan blue. For microscopic examination, stained roots were either cut into 1 cm segments or chopped into small fragments for close inspection of internal VA features. Plants not used for observation of VA fungi had shoot measurements taken and were then dried in a forced air oven at 60°C for 24 hours for dry weight determinations.

Results and Discussion

Glomus fasciculatum, G. macrocarpum, G. mosseae and the native endophytes all seem to have a similar capacity for colonizing roots of A. tridentata (Table 2). The anatomy of the VA infections of all these endophytes were similar with subtle differences that can be observed only at high magnification (Table 1). The VAM formed by G. macrocarpum, based on the structure of arbuscules, was distinguishable from G. fasciculatum and G. mosseae which were more alike. Growth of the sagebrush seedlings (Table 2) was substantially greater in all mycorrhizal plants than in nonmycorrhizal plants but no significant differences in growth were found among mycorrhizal treatments.

In order for an endophyte to be selected for use in our shrub production efforts, it must be effective under the harsh field conditions of Wyoming habitats. Further observations of VAM formed by A. tridentata and the three Glomus species will be carried out in the field.

Table 2. Height, biomass and infection frequency.

	Shoot height (cm)	Shoot dry weight (g)	Roots dry weight (g)	Infection frequency (%)
G. fasciculatum	2.5±1.1[a]*	.0054±.002[a]	.0046±.002[a]	52 ± 18[a]
G. macrocarpum	2.8±1.6[a]	.007±.002[a]	.0052±.002[a]	45 ± 14[a]
G. mosseae	2.6±1.5[a]	.0056±.003[a]	.005±.002[a]	41 ± 15[a]
native endophytes	2.1±1.8[a]	.004±.002[a]	.0066±.003[a]	38 ± 18[a]
Control	.9± .3[b]	.0009±.0002[b]	.0018±.001[b]	0

*Different superscripts indicate statistical difference at 95% confidence.

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Table 1. Comparison of endophytes.

	External hyphae	Internal hyphae	Vesicles	Arbuscules	Comments
Glomus fasciculatum	abundant 2.7-7.5 µm in diam. numerous sm. spores 25-65 µm in diam.	abundant 2.5-9.0 µm in diam. thin-walled less than .1 µm in diam.	none observed	common large, thin-walled hyphae fill cortical cells; with little branching	large coarse hyphae densely colonizing infected root areas
G. macrocarpum	less than abundant 2.5-7.0 µm in diam.	less abundant 2.0-6.0 µm in diam. thin-walled	not uncommon 5-25 µm in diam.	common highly-branched, fine hyphae densely packing cortical cells	arbuscules distinct from other 2 Glomus species also; finer hyphae
G. mosseae	less than abundant 2.5-7.5 µm in diam.	abundant 2.0-8.5 µm in diam. thin-walled	none observed	common large, thin-walled hyphae fill cortical cell; some branching	long expanses of internal hyphae with few or no associated arbuscules
Native endophytes	abundant 1.5-9.0 µm in diam. few spores	abundant 1.5-9.0 µm in diam.	rare 10-15 µm in diam.	common both fine and coarse arbuscules	roots infected by more than one endophyte

EFFECT OF SOIL-APPLIED OIL SHALE PROCESS WATER ON VA FUNGI

By

Peter D. Stahl and S. E. Williams

Keywords--Oil shale process water, VA spore populations, Glomus

Introduction

Oil shale process water, a by-product of In-situ oil shale retorting, is generated in substantial amounts during oil extraction and is considered biologically hazardous. The process water used in this study, Omega-9 retort water, has been demonstrated to have toxic effects on plants and soil microfungi. Schwab and Reeves (1979) report that retorted oil shale added to soil can reduce the formation of mycorrhizal associations in plants growing in that soil.

The objective of this study has been to investigate the long-term (4 year) effects of soil-applied oil shale process water on the VA fungi in a native soil. This was accomplished by assessing the VA fungal activity at field treatment plots and by using treated field soils in a bioassay to determine VA infection potential and spore population characteristics.

Materials and Methods

Small plots in a native sagebrush-grassland were treated with different concentrations (33%, 66%, 100%) of or no Omega-9 retort water. Four years after treatment, soil and roots from the plots were collected and brought to the laboratory. With this soil, analysis of VA spore populations were made (Stahl and Christensen, 1982), the bioassay was performed and soil physical and chemical characteristics were ascertained. Roots were examined for frequency of VAM infection.

After 100 days of growth, entire plants were harvested. Samples of roots were removed, weighed and inspected for VA infection. The roots and shoots were then dried in a forced air oven at 60°C for 24 hours and then weighed. One hundred grams of soil from each pot was collected at the time of harvest for analysis of VA spore populations.

Results and Discussion

The application of Omega-9 oil shale process water to field plots has deleterious effects on the soil and organisms examined. Four years after the addition of the retort water vegetation was still sparse on 66% and 100% field plots. Despite the fact that all soil parameters monitored in this study have returned to ambient levels, results from the field and bioassay (Fig. 1 and Table 1) indicate that something is continuing to inhibit the activity of VA fungi and the growth of yellow sweetclover in the more heavily treated soils. Because Omega-9 retort water is high in total dissolved organic carbon, which include toxic compounds such as phenols, N-heterocyclics and polynuclear aromatic hydrocarbons which may be at least partly responsible for the persistent inhibitory effects of the 66% and 100% soils.

Table 1. Results from bioassay and field.

Treatment	Dry wgt roots (g)	Dry wgt shoots (g)	Infection Frequency: Oryzopsis roots at field	Infection Frequency: Melilotus roots in bioassay
Control	1.16 ± .78[a*]	.50 ± .30[ab]	89% ± 8%[a]	72% ± 22%[a]
H_2O	1.05 ± .70[a]	.53 ± .29[a]	91 ± 8 [a]	67 ± 19 [a]
33%	1.06 ± .72[a]	.44 ± .28[b]	64 ± 31 [b]	64 ± 22 [ab]
66%	.78 ± .57[b]	.43 ± .29[bc]	38 ± 26 [c]	49 ± 24 [b]
100%	.68 ± .53[b]	.36 ± .23[c]	36 + 20 [c]	54 ± 19 [b]

*Different superscripts indicate statistical difference at 95% confidence.

References

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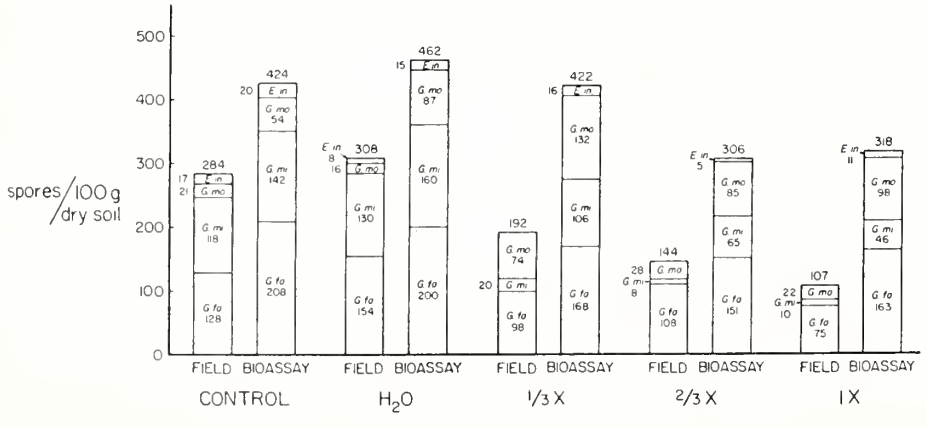
FIGURE 1
VA SPORE POPULATIONS



NATIVE MYCORRHIZAE OF SAGEBRUSH (ARTEMISIA spp.) IN S.E. WYOMING

By

D. M. H. Watson and S. E. Williams

Keywords--vesicular-arbuscular mycorrhizae, Artemisia cana, A. nova, A. tridentata vaseyana, A. tripartita

Introduction

Sagebrush (Artemisia spp.) are widespread shrubs in the semi-arid west, occurring on 15 million hectares in Wyoming alone (Beetle, 1960). The objective of this study was to observe the native vesicular-arbuscular mycorrhizae (VAM) of four sagebrush species in S.E. Wyoming: silver sagebrush (Artemisia cana); black sagebrush (A. nova); mountain big sagebrush (A. tridentata vaseyana); and threetip sagebrush (A. tripartita), and to survey the VAM fungal spore populations found in association with these sagebrush.

Methods and Materials

During the fall of 1982, sagebrush were collected from eight sites in Albany County, WY. Each sagebrush species collected at a particular site, was collected in triplicate. Soil and roots were sampled from a soil mass (ca 15 cm in diameter and 15 cm deep) surrounding the sagebrush roots. The average weight of the soil mass was about 4,000 g.

The fine roots (ca 0.1 to 1.0 mm in diameter) of the sagebrush were examined for the presence of VAM. The roots were cut into 1-cm segments, cleared in KOH, and stained in lactophenol trypan blue stain (Phillips and Hayman, 1970). Percent infection was determined as the number of root segments out of 100 that contained VAM hyphae with arbuscules and/or vesicles.

A sucrose flotation method (Allen et al., 1979) was used to extract VAM fungal spores from the soil. Spores were characterized and counted under a dissecting microscope. Identifications were made using a compound light microscope and current taxonomic keys (Gerdemann and Trappe, 1974; Hall anf Fish, 1979; Trappe, 1982). Spore numbers reported per 100 g of soil, are mean values of five 100-g soil subsamples.

Results and Discussion

The fine roots of the four sagebrush species observed, were heavily infected by VAM fungi (Table 1). Three hyphal types were noted: a winding knobby hypha, fine and coarse straight branching hyphae, and hyphae with appressoria-like thickenings at root cell penetration points. Lobed vesicles, possibly indicating infection by Acaulospora spp. were observed, as well as subglobose vesicles more typical of Glomus spp. infection. Arbuscules, peletons, and spores were also observed in the roots.

Total spore densities ranged from 495 to 2,999 spores/100 g dry soil (Table 2). Fourteen spore types were found in the Wyoming sagebrush soils: six previously reported, Entrophospora infrequens, Glomus fasciculatum, G. macrocarpum, G. mosseae, G. microcarpum, and the thick walled golden spore type (Stahl and Christensen, 1982); four tentatively identified as Gigaspora calospora, Acaulospora laevis, a Glomus macrocarpum variant (a smaller gold isolate), and a G. mosseae variant (the septum located farther down the hypha); and four new unidentified forms.

Table 1. Percent VAM infection in fine roots of four sagebrush species.

Sagebrush spp.	Range	Mean
Artemisia cana	19%-95%	63%
A. nova	61 - 97	87
A. tridentata vaseyana	52 - 97	79
A. tripartita	78 - 99	90

Table 2. VAM spore densities (total number of spores in 100 g of dry soil) from eight sagebrush sites in S.E. Wyoming.

Site	Mean density	Site	Mean density
1	495	5	2,999
2	938	6	893
3	924	7	1,613
4	508	8	580

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ENDOMYCORRHIZAE IN ACTINOMYCETE NODULATED NITROGEN-FIXING SHRUBS IN THE ROSACEAE

C. B. Perry, J. C. Stutz, and T. L. Righetti

Keywords--Cowania mexicana, Cowania subintegra, Purshia glandulosa, Pushia tridentata, Frankia

Introduction

Purshia and Cowania, rosaceous shrubs common in the Western United States, are capable of symbiotic nitrogen fixation with the actinomycete Frankia. Optimum nodulation and nitrogen fixation by Purshia appears to be limited by soil fertility constraits, even in areas with suitable Frankia populations and adequate moisture (Righetti and Munns, 1982). Although VA mycorrhizal fungi have been reported to be associated with Purshia tridentata (Williams and Aldon, 1976, and Rose, 1980), their effect on the nodulation of these shrubs is not known.

Our objective was to evaluate four species, Cowania mexicana, Cowania subintegra, Purshia glandulosa, and Purshia tridentata and a hybrid (C. mexicana X P. glandulosa), from the White Mountains in Arizona, for mycorrhizal infection, root and shoot growth and root nodulation in a soil mix from natural Cowania communities and in a modified mix with low phosphorus levels.

Methods and Materials

Soils were collected from five C. mexicana communities at sites in northern Arizona, mixed to form a composite sample, air dried, and sieved (5mm) prior to adding 1 equal part vermiculite. The population of VA mycorrhizal fungi in the composite soil was determined to be 80 spores/100 g soil. Seeds were soaked for 48 hr in aerated distilled water and then surface sterilized. Prior to planting in vermiculite, the seed coat was nicked or removed. Seedlings were transplanted at 3 weeks of age into the composite soil mix. In a second experiment, 20% limonite was added to the soil mix prior to transplanting. The phosphorus level of this treatment, determined using P isotherms, was 0 ppm.

Plants were harvested after 7 months for the first experiment and after 4 months for the low phosphorus experiment. Shoot fresh and dry weights, root fresh and dry weights, nodule fresh weight and nodule number were measured. Ten 2-cm root pieces per plant were excised, stained with acid-fuchsin lactic acid (Kormanik, 1980) and the percentage of root length colonized calculated. Analysis of variance (P=0.05) was performed after transformation of the raw data when required.

Results and Discussion

Significant differences existed between Cowania and Purshia species with respect to percent roots colonized by mycorrhizal fungi, root/shoot ratio, and number of nodules in both the soil mix and the low phosphorus soil mix. P. tridentata and C. subintegra appear to be more mycorrhizal and have a more favorable root/shoot ratio than the other shrubs in the untreated soil mix (Table 1), but in the low phosphorus treatment only the White Mountain hybrid had a relatively high root/shoot ratio (Table 2). The percentage of roots colonized by mycorrhizal fungi in the low phosphorus treatment was equal to or greater than that of the untreated soil mix.

Except for C. mexicana, plants grown in the soil mix did not differ from those in the low phosphorus soil in the number of nodules/plant. The low number of nodules formed by P. tridentata was unexpected because previous reports found that it formed more nodules than P. glandulosa in low phosphorus soils collected from Purshia communities (Righetti and Munns, 1982). This nodulation difference could be due to variability in plant genotype or to variation in the endophytic population.

Table 1. Differences between Purshia and Cowania seedlings in growth, mycorrhizal infection and nodulation.

Plant Species	Root/Shoot Ratio	Root Colonized (%)	Nodule Number (per plant)
P. tridentata	.90	34	0.3
P. glandulosa	.61	22	6.8
C. mexicana	.68	15	10.5
C. subintegra	.85	49	2.8
Hybrid	1.00	24	3.3

Table 2. Differences between Purshia and Cowania seedlings in low phosphorus soil in growth, mycorrhizal infection and nodulation.

Plant Species	Root/Shoot Ratio	Root Colonized (%)	Nodule Number (per plant)
P. tridentata	.54	39	0.4
P. glandulosa	.55	34	4.3
C. mexicana	.44	25	3.0
C. subintegra	.55	44	1.8
Hybrid	.92	43	3.6

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EFFECTS OF SOIL PARENT MATERIAL ON MYCORRHIZAL INFECTION OF ALPINE PLANTS

By

R. K. Antibus and P. C. Lesica

Keywords--calcareous, crystalline, fellfield, MPN

Introduction

Alpine ecosystems are generally characterized by soils low in available plant nutrients. It has been suggested that under these conditions plants capable of forming mycorrhizal associations have an adaptive advantage. However, little information is available concerning the mycorrhizal status of alpine plants in North America.

Our study objectives were to determine the mycorrhizal status of dominant species of alpine fellfield communities and to compare the degree of mycorrhizal colonization of species common to soils derived from calcareous and crystalline parent materials. Calcareous soils are generally characterized as being low in plant-available phosphorus, and many workers have shown a relationship between phosphorus levels and vescicular-arbuscular mycorrhizal (VAM) infection. We therefore hypothesized that greater development of VAM infection would be associated with growth on calcareous soils. To test this hypothesis, we measured VAM infection intensity of plant species growing on soils derived from both parent materials.

Methods and Materials

Six sites were sampled in Montana and adjacent Wyoming during 1982. All sites were above timberline on windswept ridges and would be classified as cushion-plant communities or fellfields (1). Study sites were separated into two groups on the basis of soil parent materials. The soils on three sites were derived from calcareous sedimentary materials, while the remaining three sites were typified by soils derived from acidic crystalline parent materials.

At each site, a 30 m transect was established perpendicular to the slope. Individual plant species cover was measured in ten quadrants, and nine soil samples were collected along the transect line. Three plants of each common species were collected along the transect. Root systems were washed and fixed in FAA in the field.

Percent mycorrhizal infection was estimated for each species on six randomly selected root sections cleared and stained with trypan blue. VAM propagule density was estimated by the MPN technique (2). Soil pH, organic matter and particle size distribution were determined by standard procedures. Soil elemental analyses were performed on 0.5 N ammonium acetate extracts using an ICP spectrometer.

Results and Discussion

Thirty-two species in fifteen families were examined for the presence of mycorrhizae. Twenty-seven species produced VAM, two were ectomycorrhizal and three were non-mycorrhizal. Haselwandter and Read (3) have reported VAM infection to be common above timberline in the Central Alps. Where comparisons between species and genera are possible, our findings concerning mycorrhizal status confirm those of Haselwandter and Read.

Multiple range testing was employed to compare the average percent mycorrhizal infection of species found at four or more sites. Of thirty-nine possible comparisons between conspecifics on different soil types, thirty had significantly higher levels of infection on calcareous soils.

Values for viable VAM propagule densities were similar to those reported from low elevation sites. Propagule numbers were not highly correlated with parent material, but were significantly correlated with phosphorus and plant cover.

Soils derived from calcareous parent materials differed from crystalline soils in texture. Calcareous soils were characterized by higher pH values and greater concentrations of several nutrients. Levels of phosphorus, nitrogen and potassium were not significantly different between the two types of parent materials.

Our data suggest that VAM infection intensity of fellfield plants was greater on calcareous than on crystalline soils. Experimental data are needed before we can determine which soil physical and/or chemical factor(s) are responsible for these observations.

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STRUCTURE AND FUNCTION OF MYCORRHIZAL FUNGUS COMMUNITIES OF THE RED DESERT

By Anne-Cressey McGraw and R. Michael Miller

Keywords--model, cost-benefit, Glomus monosporum/mosseae polymorphism

Introduction

A chronological study since 1977 in the Red Desert of Wyoming is enabling examination into the sequence of events coupling mycotrophy with revegetation following strip-mining activities. Observational data support the hypothesis that diversification in the plant community and structure is ensured with the establishment of the mycotrophic habit; thereby increasing resiliency of the community for recovery from perturbations.

The objectives of this research were: 1) to identify the species of vesicular-arbuscular mycorrhizal fungi (MF) present and evaluate primary edaphic factors affecting the population dynamics of the MF; 2) to isolate native species of MF; and 3) to develop a model system for characterizing stresses such as extremes in moisture and temperature on the coupling and function (particularly the cost-benefit of mycotrophy to the host) of mycorrhizal symbionts.

Methods and Materials

Two genera (Acaulospora, Glomus) and seven species of MF were identified from the field site: A. laevis, A. spinosa, G. etunicatum, G. fasciculatum, G. macrocarpum, G. microcarpum, G. monosporum/mosseae; however, Glomus fasciculatum and G. monosporum (GMONO)/mosseae (GMOSS) (morphpologically distinct taxa but difficult to speciate) were codominants by 1983 and were selected for the functional studies. Spore collections for each of two 'isolates' of GMONO and GMOSS from undisturbed (UN) or disturbed (stored-applied topsoil with added moisture from snow-entrapping animal exclusion fence [EC]) field treatments were used to propagate inoculum for the studies. Several significant associations were found between native plant species and mycorrhizae at the mine site (Table 1). From these associations Agropyron smithii Rydb. was selected for the model text plant. Additionally seeds of two varieties ('Ariba' and 'Rosana', originating from Colorado and Montana, respectively) of Ag. smithii were utilized in the studies. Grass transplants were placed in 165-mℓ plastic tubes filled with 200 g of fumigated, aerated sand:soil (Terrada series - coarse, sandy loam) (2:1, v/v) medium which was either noninfested or infested separately with each of four isolates of MF to achieve 0.1 spore/g dry wt of soil. Plants were grown in a growth chamber (545 $\mu E/m^2/s$, 14 L/D), and six replicates for each var. x isolate combination were sacraficed at 10 wks after transplanting for evaluating above- and below-ground plant growth and fungus activity.

Results and Discussion

Growth and morphology differed for both plant var. with respect to inoculation and for fungus x var. combinations (Table 2). Rhizome production (and to some extent tillering) and root biomass were greater and lesser, respectively, in plants inoculated with MF compared with uninoculated plants. Sporulation occurred in significantly higher numbers in 'Rosana' with GMONO-UN than with any other var. x isolate combination. Additionally, sporocarps of GMONO-UN were observed in 'Rosana'; whereas, root colonization and extramatrical mycelia were more extensive in 'Ariba'. Fungus activity ceased with a single rock phosphate amendment, where the level of sodium bicarbonate-extractable phosphorus (PO_4) ≥ 75 µg PO_4/g. In a parallel experiment, weekly amendments of phosphate (12.8 µg PO_4/g total) added in nutrient solution to soil-containing growth media (20 µg PO_4/g initially) diminished but did not eliminate fungus activity. In future studies a PO_4-containing nutrient solution will be used for fertilization. The increased rhizome production (and to some extent tillering) and decreased root growth in several isolate x var. combinations is interpreted to mean that an alternate growth strategy can be practiced by mycorrhizal plants. Verification of this hypothesis will be attempted in the Wyoming site. Root colonization rarely exceeds 30% in field-collected roots or when native fungus isolates and plant species are combined. Instead a polymorphic switch occurs toward increased development of extramatrical mycelia. The cost of mycotrophy to the host may be reduced with this polymorphism. True (single-spore origin) isolates of GMONO-UN and GMOSS-UN established from the cultured inoculum used in this study have been obtained and will aid in future studies characterizing the range in cost-benefit to the host.

TABLE 1. ASSOCIATIONS (CORRELATION COEFFICIENT, R) BETWEEN COMMUNITY TYPE AND MYCORRHIZAE FOR UNDISTURBED (UN) AND DISTURBED (DA) LAND.

VEGETATION TYPE	TOTAL VIABLE-APPEARING SPORES UN	TOTAL VIABLE-APPEARING SPORES DA[*1]	EMPTY SPORES UN	EMPTY SPORES DA	G. FASCICULATUM UN	G. FASCICULATUM DA	G. MONOSPORUM/MOSSEAE UN	G. MONOSPORUM/MOSSEAE DA[*2]
GRASSES	NS[*3]	0.22*[*4]	NS	NS	NS	0.22*	0.56*	NS
ATRIPLEX CONFERTIFOLIA	NS	NS	NS	-0.28*	NS	0.23*	NS	NS
HALOGETON GLOMERATUS	NS	-0.44***	NS	-0.29**	NS	-0.38***	NS	-0.37***

[*1] TREATMENT INCLUDES DIRECT-APPLIED AND STORED-APPLIED TOPSOIL AS DISTURBANCE FACTORS.
[*2] G. MONOSPORUM/MOSSEAE SPORE POPULATION DENSITY LEVELS SIGNIFICANTLY CORRELATED WITH GRASSES IN DIRECT-APPLIED BUT NOT STORED-APPLIED TREATMENT.
[*3] CORRELATION COEFFICIENTS NOT SIGNIFICANT (NS).
[*4] CORRELATION COEFFICIENTS SIGNIFICANT AT P < 0.05, 0.01, 0.001 (*,**,***, RESPECTIVELY).

TABLE 2. ACTIVITY OF NATIVE ISOLATES OF MYCORRHIZAL FUNGI IN RELATION TO GROWTH OF TWO VARIETIES OF AGROPYRON SMITHII.

VARIETY	FUNGUS ISOLATE	PLANT GROWTH: TILLER NO./PLANT	PLANT GROWTH: RHIZOME NO./PLANT	PLANT GROWTH: ROOT DRY WT/(MG)	MYCORRHIZAL FUNGUS ACTIVITY: ROOT LENGTH COLONIZED (%)	MYCORRHIZAL FUNGUS ACTIVITY: (M)	MYCORRHIZAL FUNGUS ACTIVITY: SPORULATION INDEX[*1]
ARIBA (COLORADO)	GMONO-UN[*2]	3.55 B[*3]	0.73 AB	638 B	15.3 A	44.4 A	0.17 B
	GMONO-EC	4.64 A	0.18 C	546 B	2.2 BC	6.2 B	0.00 B
	GMOSS-UN	3.90 AB	0.80 A	571 B	7.3 ABC	22.8 B	0.17 B
	GMOSS-EC	3.75 AB	0.75 AB	586 B	7.9 ABC	18.2 B	0.00 B
	NONINOC.	3.73 AB	0.18 C	817 A	0.0 C	0.0 B	0.00 B
ROSANA (MONTANA)	GMONO-UN	2.42 B	0.17 C	469 B	10.6 AB	17.8 B	0.83 A
	GMOSS-UN	3.58 B	0.25 BC	473 B	2.8 BC	6.0 B	0.33 B
	NONINOC.	3.91 AB	0.09 C	463 B	0.0 C	0.0 B	0.00 B

[*1] BASED ON INDEX OF 0-4, WHERE 0 = NO SPORULATION ON ROOTS, 4 = ABUNDANT SPORULATION.
[*2] ORIGIN OF ISOLATES; UN = UNDISTURBED, AND EC = STORED-APPLIED TOPSOIL PLUS SNOWFENCE.
[*3] COLUMNS OF VALUES FOLLOWED BY DIFFERENT LETTERS DIFFER SIGNIFICANTLY (P < 0.05) BY A PROTECTED LSD PROCEDURE.

EFFECT OF SOIL DILUTION ON THE GROWTH AND RESPONSE OF AGROPYRON SMITHII TO MUTUALISTIC ASSOCIATION WITH GLOMUS MOSSEAE/MONOSPORUM

By: A. G. Jarstfer and R. M. Miller

Keywords--Agropyron smithii, tiller, phosphorus, growth strategy, Glomus mosseae/monosporum

Introduction

Studies on morphological response of rangeland grasses to mycorrhiza are limited with studies addressing the interaction between morphology and physiology being almost non-existant. The research presented is part of an overall study aimed at understanding the relationship between host and endophyte for rangeland plants.

Materials and Methods

Ninety Agropyron smithii Rybd. (cv. Rosana) plants were grown with a viable or sterlized inoculant of Glomus mosseae/monosporum mix in a soil dilution series with quartz sand. Seeds of A. smithii were germinated in perlite and transplanted to 200g growth media when 5 cm in height. Five soil dilutions on a vol./vol. basis (4:1, 2:1, 1:1, 0:1, and 0:1 + soil wash) of a coarse sandy loam, 'Terrada series' soil to sand (20-40 mesh) were made to achieve soil (P) levels of 20, 15, 12, 2+W, 2 µg . g^{-1} (Table 1). A balanced design consisting of 5 soil dilutions, 2 inoculation and 9 replicates (5 x 2 x 9) was used to analyze the data. G. mosseae/monosporum soil-root inoculum was added to achieve 0.5 spores per gram media. Autoclaved inoculum and an inoculum wash were added to uninoculated treatments. Ten mℓ nutrient solution minus P was added weekly and plants were watered to 70% field capacity with DI water every third day. A P pulse was given at 6 and 7 weeks. Plants were growth in a walk-in growth chamber on a 16/8 hour L/D cycle at a quantum flux density of 470 µE. m^{-2} sec^{-1}. Temperature was 25°C/20°C and relative humidity was 60%. At ten weeks the shoot was cut at 'soil' surface and dried at 80°C for 48 hours. Roots were separated from the growth media by sonication and water wash. This process was found to be effective at removing external mycelium and spores from roots without damage to roots. Crown and rhizome were separated, dried, and weights taken. Fresh weights were taken and roots stained and observed by the gridline intersect method.

Results and Discussion

A significant soil dilution and inoculation response was found for growth and morphology characteristics of A. smithii after 10 weeks growth (Table 1). The response of A. smithii to the different soil P levels was not necessarily a linear one in that the highest levels for a particular parameter were not always associated with the highest soil:sand dilution (4:1). Plant growth was significantly influenced by increased soil P levels (Table 1).

The response to inoculation for most growth parameters was positive (Table 1). In A. smithii, mycorrhizal plants had root:shoot ratios closer to one than nonmycorrhizal plants. Mycorrhizal plants also produced greater biomass and leaf surface area than their nonmycorrhizal counterparts. Also, a significant increase in total root length was found. Until tissue nutrient levels are available an explanation for many of the differences reported is not possible.

The major finding in this study was a growth strategy change for plants possessing V-A mycorrhiza where an increase in tiller production by the mycorrhizal plants at all soil P levels was found (Table 1). However, hormone changes within the plants caused by the presence of V-A mycorrhizal fungi may be responsible for this response.

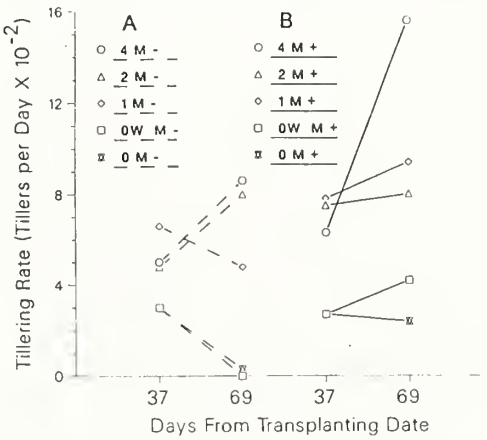
A phosphorus pulse was given to all treatments after six weeks growth in order to evaluate how A. smithii responds to a P pulse. The data presented in Fig. 1 on tillering rate suggest that mycorrhizal plants are capable of utilizing the pulsed P at all soil P levels as represented by tillering rate changes. This data suggests that mycorrhiza are advantageous to plants in order for them to respond to periodical nutrient pulses.

TABLE 1. MEANS OF VARIOUS PLANT AND MYCORRHIZA PARAMETERS FOR A. SMITHII AFTER 10 WEEKS GROWTH.

GROWTH MEDIA (PARAMETERS)††	VAM INOCUL.	INFECTION (%)	TOTAL ROOT LENGTH (CM)	TOTAL PLANT DRY WT. (MG)	SHOOT AREA (MM^2)	TILLER NUMBER	ROOT/ SHOOT RATIO
4:1 20	-	0	1729	942	11197	4.6	0.51
4:1 20	+	54.2	2111	934	12107	7.3	0.55
2:1 15	-	0	2219	882	9151	4.3	0.44
2:1 15	+	18.7	2088	1083	10363	5.3	0.50
1:1 12	-	0	1573	992	8858	4.0	0.36
1:1 12	+	28.7	1587	1111	10831	5.8	0.42
0:1 2+W	-	0	550	273	2996	1.1	0.34
0:1 2+W	+	29.3	1190	417	4028	2.3	0.52
0:1 2	-	0	759	239	2949	1.2	0.31
0:1 2	+	52.6	872	323	4743	1.7	0.45
R SQUARE		0.84	0.77	0.93	0.77	0.66	0.50
SOIL DILUTION		**†	***	***	***	***	***
INOCULATION		***	N.S.	***	**	***	***
SOIL-INOCULATION INTERACTION		**	N.S.	*	N.S.	N.S.	N.S.

†PR>F: *<.05; **<.01; ***<.001.
††SOIL DILUTION RATIO AND SOIL P CONCENTRATION (PPM).

Fig. 1. Effect of P Pulse on A. smithii Tillering Rate for Soil and Inoculation Treatments



OCCURRENCE AND CHARACTERISTICS OF VAM IN ECOLOGICALLY DIFFERENT SITES

By

A. Sabharwal and K. G. Mukerji

Keywords -- Habitat effects

Introduction

The occurrence and characteristics of vesicular arbuscular mycorrhiza was examined at different sites, in various soil types and host plants. The present study was aimed to compare and to assess the incidence of VAM and its mycorrhizal colonization in different hosts at different sites and also to ascertain the identity of endophyte based on the infection patterns.

Methods and Materials

The area for investigation was selected in the Delhi University Ridge a natural forest stand. It comprises both of protected as well as unprotected areas. The Ridge area was divided into four sites namely, A, B, C and D (D being the unprotected or the disturbed site) for collection of plant material. Throughout summer of 1982-83 at least three specimens of each of the major flora present at the chosen sites was collected. Once a plant was collected, the roots were preserved in FAA in the finest order of their branching. Soil (100-200 gms) was collected from surface to a depth of 25 cms around the lateral roots and analysed for spores, pH, texture, phosphorous, calcium and soluble salts. Standard techniques were used for isolation and spore count (Gerdemann and Nicolson, 1963; Phillips and Hayman, 1970).

Results and Discussion

The results of the investigation clearly indicated the presence of VAM in all the plants from all the sites exceptions being the members of Cyperaceae & Amaranthaceae at the disturbed sites. It was found that VAM was absent from the plants at unprotected site which were otherwise mycorrhizal at the protected sites. Even the number of spores was found to be greater at the sites A, B, C than the site D. Mycorrhizal infection varied in all the plant species ranging from 30% to 85%. Differences in the degree of infection was clearly evident in the plants from all the different sites studied. Plant species present at a relatively more disturbed site showed low degree of infection than the plant species present at the undisturbed site. All the above results can be explained on the basis of non-availability of inoculum necessary for infection due to disturbance at the disturbed sites. In the present investigation it was found that spore numbers among the plant root were generally low although levels of root infections were high. So it can be concluded that the presence or absence of VAM, and the degree of infection is also related to the particular site at which the plant species was present.

The characteristic infection pattern was observed in the roots of all the host plants studied (Fig. 1). Many of the fungi could be distinguished from each other, though at generic level.

The results of the present investigation showed that there was no apparent relationship of VAM infection to soil characteristics but they had a cummulative effect on the mycorrhizal infection at the different sites.

Fig. 1. Stelar Infection in the roots of family Gramineae. A= Dicanthium annulatum, B, C, D, E = Cynodon dactylon. LH= Looped hyphae, st= stele, s= swollen junctions.

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NATURAL RECOLONIZATION OF RECLAIMED STRIPMINE LAND BY ENDOGONACEOUS MYCORRHIZAL FUNGI

By

Ann Brooks Gould and James W. Hendrix

Keywords--reclamation, most-probable-number estimation, Glomus aggregatum, Glomus claroideum

Introduction

Stripmining involves removal of topsoil and parent material. Topsoil on land mined before 1967 usually was not saved. The absence of mycorrhizal fungi in minespoil may be a major problem in revegetation. The purpose of this study was to survey endomycorrhizal propagule densities in one mine at several sites mined in the 1940's and reclaimed from zero to nine years prior to 1984.

Materials and Methods

Orphaned stripmine sites, mined in the 1940's and reclaimed in 1974, 1978, 1981, 1982 and 1983 were divided into subsites and sampled on four dates over a one and one-half year period, beginning in May, 1982. The spoil collected was dried, sieved and serially diluted with sterile sand for bioassay with a sorghum-sudangrass hybrid. After a six week growth period, each root system was assessed for the presence or absence of mycorrhizal structures and the population density in undiluted spoil was calculated by a most-probable-number (MPN) technique. Spores of endomycorrhizal fungi were isolated using a sucrose-centrifugation method, counted and identified to species.

Results and Conclusions

Propagule and spore population densities were high in spoil reclaimed several years (since 1974 or 1978), but were low in spoil reclaimed only a few months (Fig. 1, 2). Populations in spoil reclaimed in fall, 1981 rose to high levels by June, 1983. Populations at sites reclaimed in 1982 appear to be following the same trend. Glomus aggregatum appeared earliest in reclaimed spoil, followed by Glomus claroideum. These species are seldom prominent in Kentucky agricultural soils. With time, however, species characteristic of agricultural soils, such as Glomus macrocarpum, appear.

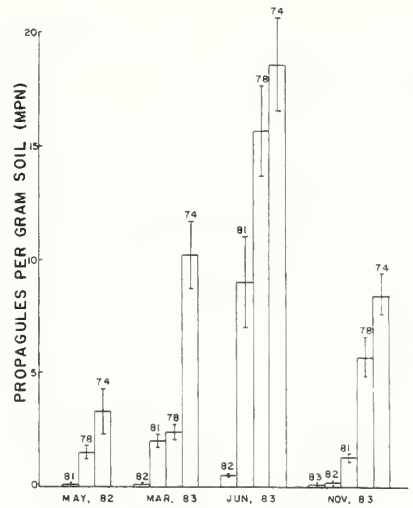

FIGURE 1

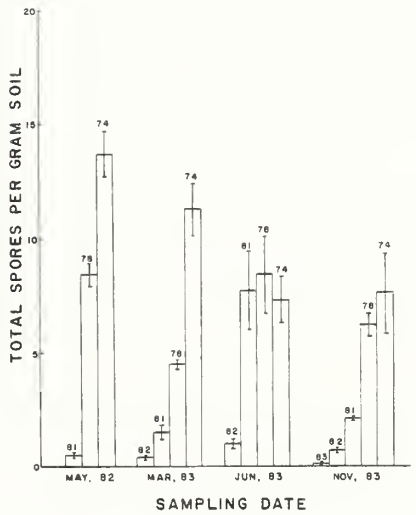

FIGURE 2

TABLE 1: MYCORRHIZAL SPORE DENSITES FOR FIELDS SAMPLED IN NOVEMBER, 1983.

	YEAR RECLAIMED				
	SPORES/G SOIL				
	1974	1978	1981	1982	1983
GLOMUS AGGREGATUM	3.8	2.0	1.0	0.4	TR*
GLOMUS CLAROIDEUM	2.9	1.6	TR	0	0
GLOMUS MACROCARPUM	0.6	1.6	0	TR	0
GLOMUS CALEDONIUM	0.1	0.1	0.8	0	0
GLOMUS FASCICULATUM	0.5	0.4	TR	TR	0
GLOMUS CONSTRICTUM	0.4	TR	0	0	0
GLOMUS MICROCARPUM	TR	0.1	TR	TR	TR
GLOMUS MOSSEAE	TR	TR	TR	TR	0
ACAULOSPORA ELEGANS	0.1	0.1	0	0	0
ACAULOSPORA LAEVIS	0	TR	0	TR	TR
GIGASPORA HETEROGAMA	0	0	0	TR	0
UNIDENTIFIED SPORES (< 35µM)	0.2	0.1	0.2	0.3	TR
TOTAL	7.6	6.2	2.1	0.7	0.1

*TRACE

VA MICORRHIZAE OF APPLES : THE INFLUENCE OF SOIL TREATMENTS AND SEASON

By

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Keywords - soil treatment, apple, VAM, season

Introduction

A series of experiences was carried out by IPLA with special regard to three aspects:
1) The occurrence of VAM in apple orchard
2) The influence of soil treatments on the occurrence and development of apple symbiosis
3) The influence of season on VAM and the presence of endomycorrhizal spores in the soil.

This paper describes the results so far obtained.

Methods and Materials

In an apple orchard of Golden Delicious c v, 3 different soil treatments were compared : bark mulch, grass mulch, mechanical and chemical weeding. Treatments were repeated every year; 8 years later samples of soil and apple rootlets were collected at 4 depth levels of soil, in different seasons.
Spore numbers were evaluated by Mosse and Bowen method (1), in the 70μm-420μm soil fraction. Mycorrhizal incidence was evaluated as described by Nappi et alii (2).

Results and Discussion

Spore numbers were high in the top layers of soil (up to 472 spores/g in bark mulch) and decreased with depth (Fig. 1). The soil with mulched and permanent grass had far more spores (the annual averages of spore values were 195/g and 66 spore/g, respectively) than the weeded ones (average = 49 spore/g).
Spore numbers were highest in May, decreased from spring to summer with lowest values in July and later increased progressively.
Mycorrhizal incidence showed relatively high values without remarkable differences between treatments : annual average = 84% in bark mulch, 74% in grass mulch, 73% in weeded soil. Moreover the values were high in all depth levels; no differences throughout the whole vegetative year were remarked. Mycorrhizal incidence on the other hand showed marked seasonal differences : it was lowest in May and increased from July to January (Fig. 2).
The results obtained have shown a remarkable influence of soil treatments on spore presence : in fact bark mulch and grass mulch lead to higher spore numbers in the soil, probably due to higher soil organic matter content.

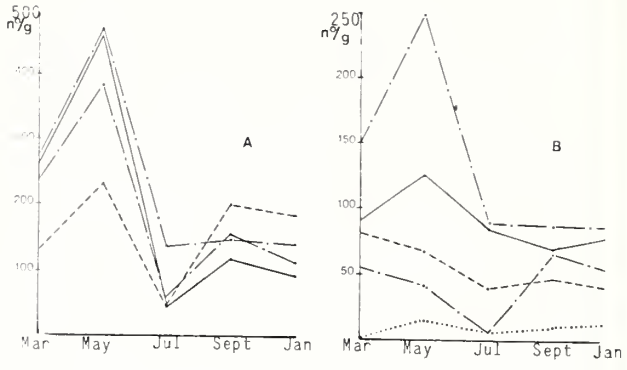
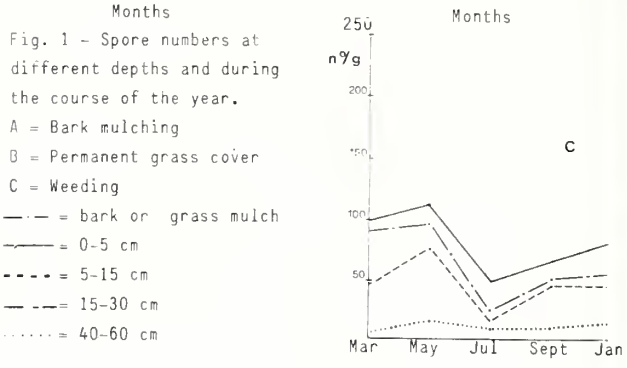


Fig. 1 - Spore numbers at different depths and during the course of the year.
A = Bark mulching
B = Permanent grass cover
C = Weeding
— · — = bark or grass mulch
——— = 0-5 cm
- - - - = 5-15 cm
— — — = 15-30 cm
····· = 40-60 cm



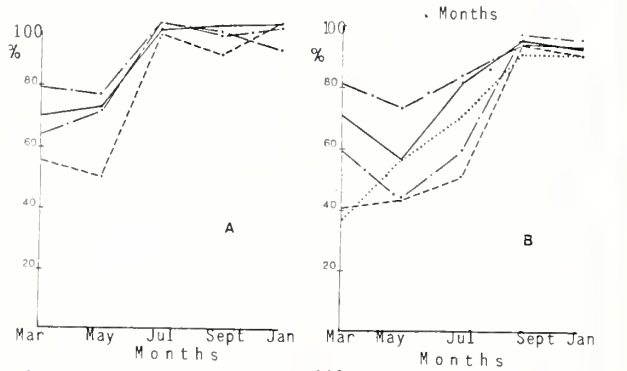
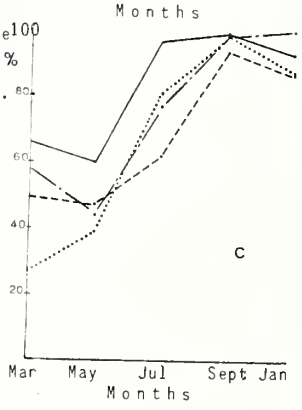


Fig. 2 - Mycorrhizal incidence at different depths and during the course of the year.
A = Bark mulching
B = Permanent grass cover
C = Weeding
— · — = bark or grass mulch
——— = 0-5 cm
- - - - = 5-15 cm
— — — = 15-30 cm
····· = 40-60 cm



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MYCORRHIZAS & INTER-PLANT TRANSFER OF NUTRIENTS. I. ECTOMYCORRHIZAS.

By

D. J. Read and R. D. Finlay

Key words: Ectomycorrhiza, mycelial interconnections, carbon transfer, phosphorus transfer

Introduction

The physiology of ectomycorrhizal roots has been extensively studied (see Harley & Smith 1983) but less is known of the delicate external mycelial systems which grow from infected roots to exploit soil. The biology of such systems can only be examined realistically by using non-destructive techniques. Simple methods first employed by Skinner and Bowen (1974) have now been further developed to investigate the structure and function of ectomycorrhizal mycelia formed by selected fungal symbionts in association with host plants grown in intra- and interspecific combination in root chambers.

Materials and Methods

Mycorrhizas are first synthesized under aseptic conditions using peat-vermiculite and modified Melin-Norkrans medium. The infected plants are then transferred to transparent chambers containing natural forest soil. Uninfected seedlings of the same or of another host species may be introduced into the chamber at the same time. The range of host-fungus combinations so far employed is shown in Table 1. The progress of extension of the mycelium from infected roots into the soil and then onto uninfected roots can be analysed, and isotopic tracer techniques used to investigate patterns of nutrient and water movement within the system. Carbon transfer has been examined after exposure of shoots of 'donor' plants to $^{14}CO_2$ in sealed chambers (Brownlee *et al.* 1983). Water and phosphorus transfer is followed after placing terminal parts of mycelial strands into tritiated water (Duddridge *et al.* 1980), or ^{32}P labelled orthophosphate solutions. Autoradiographic methods are used for qualitative determination of transfer processes in the undisturbed chambers, and quantitative estimation of isotope distribution is achieved after harvesting all seedlings in the chamber, digesting their component parts in NCS tissue solubiliser and determining radioactivity in a liquid scintillation counter.

Results

Mycelia grow from mycorrhizal roots in fan-like formations which provide effective soil exploitation. When the leading edge of a mycelial fan makes contact with an uninfected root, surface colonisation takes place. If contact is with a lateral root at the appropriate stage of development mycorrhiza formation occurs. Because of the low levels of host specificity shown by many ecto-mycorrhizal fungi the infection processes can be observed in both inter- and intra-specific combinations of host plants (Table 1). This pattern of inoculum spread inevitably results in the presence of mycelial interconnections between individual plants and between those species with which a given fungus is compatible. Carbon is also readily transferred between combinations of interlinked plants (Table 1) as shown by autoradiography and by subsequent quantitative analysis (Table 2). Label accumulates in the sheath of receiver roots and is later transferred to the shoots. Prolonged shading of receiver plants leads to the accumulation of significantly more label in the seedling roots but to lower activity in the shoots (Table 3). In these conditions over 6% of donor carbon can be transferred to the receiver.

Phosphorus applied to the distal parts of the mycelial system moves freely through the anastomosing network of strands to all infected seedlings of a chamber (Plates 1a and 1b). Label accumulates first in the mycorrhizal roots but then moves rapidly to the shoots (Plate 1b). Again, isotope moves readily to seedlings of a number of species interconnected by a common mycorrhizal mycelium. This pattern of distribution is comparable with that observed by Duddridge *et al* (1980) using tritiated water.

Discussion

Mycelial systems of ecto-mycorrhizal fungi are shown to act as sources of inoculum which give rise to inter-plant connections at both intra- and inter-specific level. Studies of Fleming (1983) suggest that the infection process follows this pattern in natural forest situations.

The interconnections provide channels through which carbon, phosphorus and water can be transferred along gradients of potential between the linked individual plants. The broader physiological and ecological implications of these observations are discussed in the following paper.

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Table 1. Host and fungus combinations in which inter-plant mycorrhizal connections have been synthesized and $^{14}CO_2$ transfer demonstrated (+). Cases where mycorrhizas have not formed in the 'receiver' are indicated with zero (0). Dash (-) indicates synthesis not attempted.

Donor Species	Receiver Species	Fungal Species					
		Amanita muscaria	Rhizopogon roseolus	Paxillus involutus	Suillus granulatus	Suillus bovinus	Suillus luteus
Pinus sylvestris	Pinus sylvestris	+	+	+	+	+	+
	P. contorta	+	+	+	+	+	+
	Picea abies	+	+	+	0	0	0
	P. sitchensis	+	0	+	0	-	-
	Betula pubescens	+	+	+	0	0	0
Pinus contorta	Pinus contorta	+	+	+	+	+	+
	P. sylvestris	+	+	+	+	+	+
	Picea abies	+	+	+	0	0	0
	P. sitchensis	+	+	+	0	0	0
	Betula pubescens	+	+	+	0	0	0

Table 2. The distribution of radioactivity (d.p.m. per mg dry wt.) in roots and shoots of mycorrhizal (M) and non-mycorrhizal (NM) Pinus contorta plants grown in association with 'donor plants' of the same species.

	Treatments		
	NM	M (Suillus bovinus)	M (Suillus granulatus)
replicates	(n=4)	(n=8)	(n=8)
Variable			
Shoot d.p.m. mg^{-1}	98	305 (P < 0.05)	157 (n.s.)
Root d.p.m. mg^{-1}	304	3641 (P < 0.001)	1639 (P < 0.05)
Activity in whole receiver plant as % of that in donor	0.028	0.23 (P < 0.001)	0.25 (P < 0.05)

Donor plants were fed with 50 µCi of $NaH^{14}CO_3$ for 72 hours.

Significance levels refer to differences between NM and M treatments and are based on analysis of variance of ln transformed d.p.m. data.

Table 3. The distribution of radioactivity (d.p.m. per mg dry wt.) in shoots and roots of unshaded (NS) and shaded (S) Pinus contorta plants grown in association with 'donor' plants of the same species. All plants were infected with the ectomycorrhizal fungus Suillus bovinus.

	Treatments		
	NS		S
replicates	(n=8)		(n=9)
Variable			
Shoot d.p.m. mg^{-1}	749	(P < 0.01)	25
Root d.p.m. mg^{-1}	11129	(P < 0.01)	21527
Activity in whole receiver plant as %	3.8	(n.s.)	6.4

Donor plants were fed with 50 µCi of $NaH^{14}CO_3$ for 120 hours.

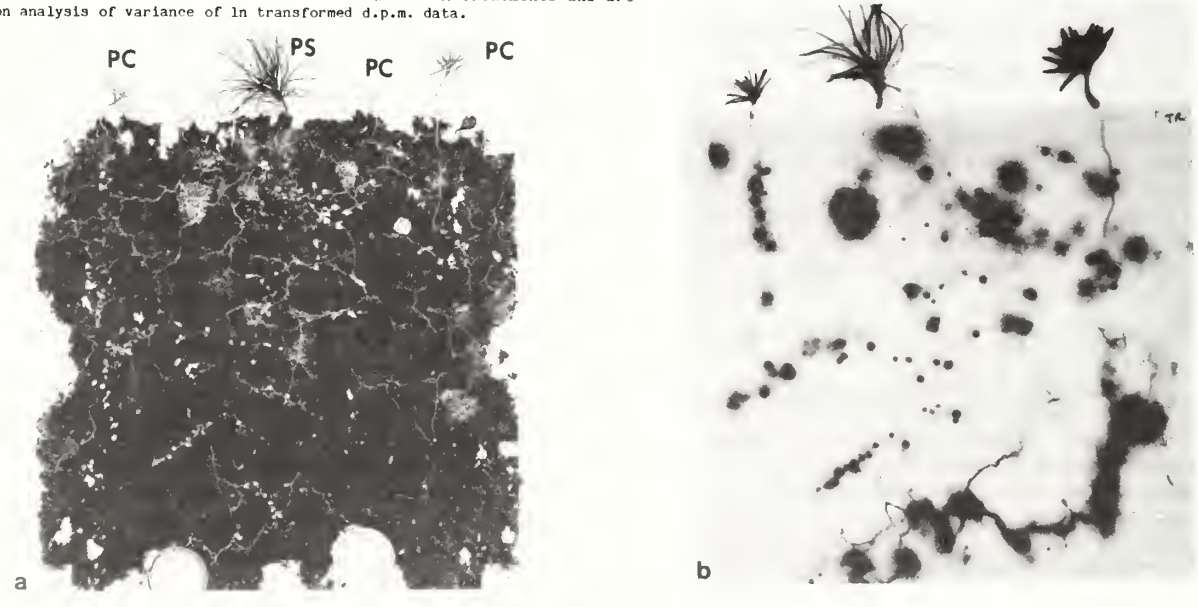


Plate 1 (a) Pinus sylvestris (PS), the infection donor, and P. contorta (PC) interlinked by mycelial strands of Suillus bovinus which are being fed with 50 µCi ^{32}P. (b) Autoradiograph of chamber after 6 days.

MYCORRHIZAS AND INTER-PLANT TRANSFER OF NUTRIENTS. II. VESICULAR-ARBUSCULAR MYCORRHIZAS

By

D. J. Read and R Francis

Key words: VA mycorrhiza, mycelial interconnections, carbon transfer, nutrient transfer

Introduction

In grassland as in other types of natural vegetation dominated by plants with VA mycorrhizas, infection of individual plants arises largely through contact between uninfected roots and the mycelial network growing from established plants in the community (Read et al 1976). This mode of infection is comparable with that seen in ectomycorrhizal plants (Read and Finlay, previous abstract) and it is therefore important to determine whether the consequences of the infection process are comparable in both types of mycorrhiza.

Materials and Methods

Experiments have been designed to examine the role of VA mycelial interconnections. In order to establish infection in mycorrhizal (M) systems, plants to be employed as 'donors' of infection and nutrients are first inoculated with spores or root fragments. When infection has occurred, the M donor plants are transferred to culture vessels containing partially sterilised dune sand into which uninfected receiver seedlings are planted. Parallel systems containing uninfected (NM) donor plants are also established. The following types of experimental systems have been employed:

(a) Transparent dish systems. Donor and receiver plants are grown together in plastic Petri dishes until infection is observed in M receiver seedlings. Some receivers are then shaded and the shoots of M and NM donors are exposed to $^{14}CO_2$ for 24 h after which time the donor shoot is excised and sand particles are carefully removed. The exposed mycelial network is photographed and the distribution of tracer analysed by stripping film autoradiography. Quantitative determination of isotope transfer is later carried out using methods employed for ectomycorrhizas. Plantago lanceolata is used as donor and Festuca ovina as receiver because their roots can readily be distinguished on the basis of size in photomicrographs.

(b) Simulated sward systems. Single infected or uninfected donor plants of Plantago are transferred to a central position in a seed tray (38 x 24 cm) containing irradiated sand. Receiver seedlings of Plantago and Festuca are planted around the donor with gaps of 6 cm between individual plants. After 8 weeks, during which time infection spread from donor to receiver plants in the M series, half of the receivers in each sward are placed under an aluminium foil shade and the shoot of the central 'donor' plant is exposed to 100 µCi of $^{14}CO_2$. After 48 hours all plants are harvested for determination of total radioactivity.

(c) Double pot system. The root system of M and NM donor plants is divided so that approximately half of the roots are placed into each of two adjacent pots (A & B). Receiver plants are placed in one pot (B) and nutrient treatments are applied to pot A (Fig. 1). The combination of donor and receiver plants is Plantago - Plantago + Arabis, Plantago - Festuca + Arabis, Festuca - Festuca + Arabis. The cruciferous plant Arabis hirsuta is normally not susceptible to infection and is included as a control. Receivers are grown for 40 days during which time those in the M system became infected. They receive only water during this period. An initial harvest at this point is followed by application of nutrient solution to A for the rest of the experiment. Further harvests of receiver plants are taken at 84 and 126 days.

Results

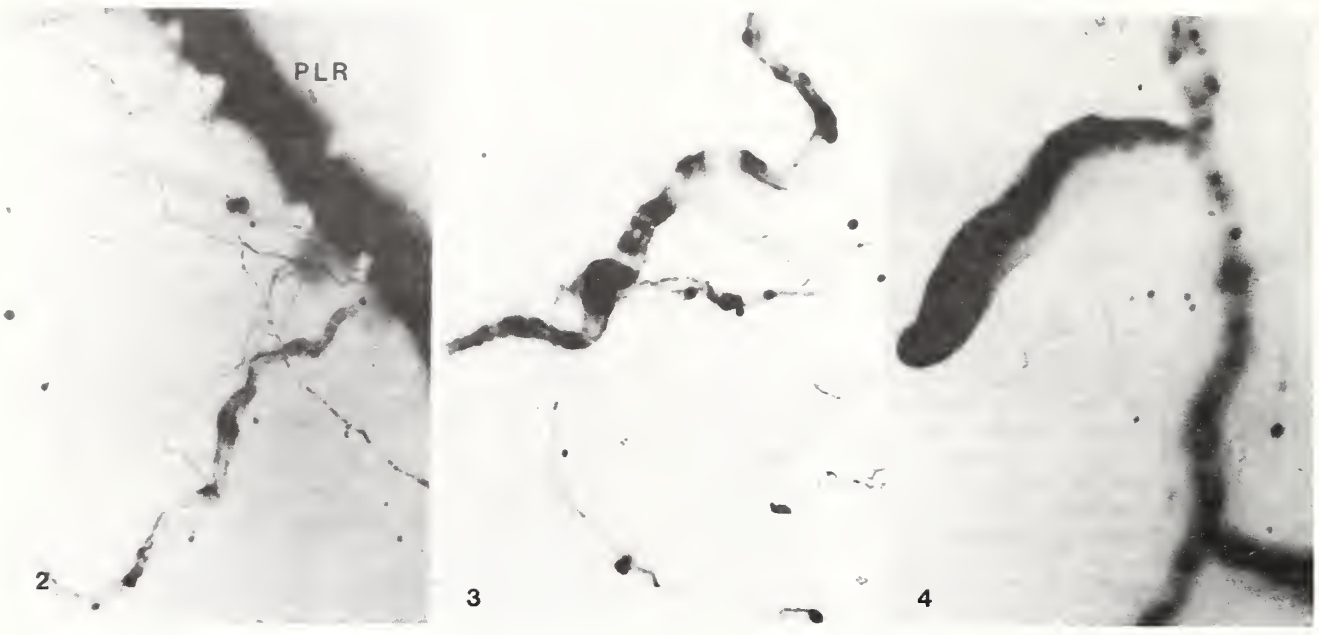
Microscopic and microautoradiographic analysis of the transparent dish systems reveals that infection spreads from Plantago donors to infected Festuca receivers and that a network of direct hyphal interconnections arises through which ^{14}C moves readily from donor to receiver plants. Label is first detected in the mycelial network (Fig. 2), then in intracellular fungal structures in the host (Fig. 3) and finally it accumulates in root apices (Fig. 4) and shoots. The quantity of carbon transfer is strongly influenced by shade (Table 1). Little or no transfer occurs in NM system and there is no evidence of significant leakage of label from infected or uninfected roots (Francis and Read 1984).

In sward systems carbon moves to all mycorrhizal receiver seedlings while little label is transferred between NM plants. (Figs. 5 and 6). Again, shading causes an increase of activity in the M plants. Mycelial interconnection thus provide channels for transport to numerous plants growing together in the sward.

After 40 d in double pots, infection levels were 25-35% in Festuca and Plantago and zero in Arabis. No significant growth responses were recorded in this period (Fig . 7.) Following nutrient application to compartment A, mycorrhizal receiver seedlings produced significantly greater yields than their non-mycorrhizal counterparts in all but one case at both the 84 and 124 day harvests. Arabis shows a significant growth response at one harvest only. Mycorrhizal infection thus facilitates transfer of nutrients from Compartment A, by way of the M donor plants, to connected receivers in compartment B.

Discussion

The experiments confirm that as in ectomycorrhizas, VA mycorrhizal infection spreads by hyphal extension from root to root. The resulting hyphal bridges provide channels for transfer of nutrients between plants. Carbon moves along gradients of potential from plant to plant by the direct hyphal pathway and facilitated uptake appears to be unimportant. It is likely that phosphorus, which has also



Figs. 2.3.4. Stripping film autoradiographs of mycorrhizal system taken 24 h (Fig. 2), 48 h (Fig. 3) and 72 h (Fig. 4) after feeding donor Plantago roots (PLR) with $^{14}CO_2$.

Table 1. Radioactivity in shoots and roots of receiver plants (Festuca ovina) grown with Plantago donors under three light regimes. Counts are expressed as d.p.m. mg dry weight (with 95% confidence limits) and as percentage of the total activity in the donor plants at harvest. (n = 6). ** P = < 0.05, * P = < 0.01. (From Francis & Read, 1984).

Treatment	Receiver category: Festuca Mycorrhizal			Festuca Non-Mycorrhizal		
	Activity in Root dpm/mg dry wt	Activity in Shoot dpm/mg dry wt	Activity in whole receiver plant as % of that in donor	Activity in Root dpm/mg dry wt	Activity in Shoot dpm/mg dry wt	Activity in whole receiver plant as % of that in donor
Full light	$8908^{**} \pm 3530$	$363^{*,ns} \pm 242$	0.0151	$656^{ns} \pm 403$	$147^{ns} \pm 194$	0.0019
$^1/_2$ light	$18072^{**} \pm 7647$	$479^{*,ns} \pm 75$	0.048	$264^{ns} \pm 365$	$229^{ns} \pm 372$	0.0010
Dark	$57218^{**} \pm 12372$	$51^{ns} \pm 38$	0.112	$171^{ns} \pm 100$	$nd^{*,ns}$	0.0005

been shown to pass freely from plant to plant (Heap and Newman 1980, Whittingham and Read 1982, Chiariello et al 1982), moves by the same pathway. Movement of nutrients occurs in sufficient quantities to promote growth responses in receiver plants. Since the transfer process occurs in a similar manner in both VA and ectomycorrhizas it is likely to be of comparable importance in both mycorrhizal types. This importance can be assessed at three levels:

1. The individual plant. In natural vegetation seedlings frequently become established in situations where light and nutrient supplies are extremely limited. Rapid integration into a network of hyphal interconnections may enable them to be sustained during their establishment, assimilates and mineral nutrients being transferred from well illuminated mature plants.

2. The plant community. While improved survival potential in individual plants may have provided selection pressure in favour of the mycorrhizal habit, similar pressures would favour low host specificity in the fungus because this increases the chance that the heterotroph finds a suitable resource base. A secondary, but nonetheless important, consequence of these two effects is that many intra and inter-specific hyphal connections occur between plants in nature. Grime (1973),

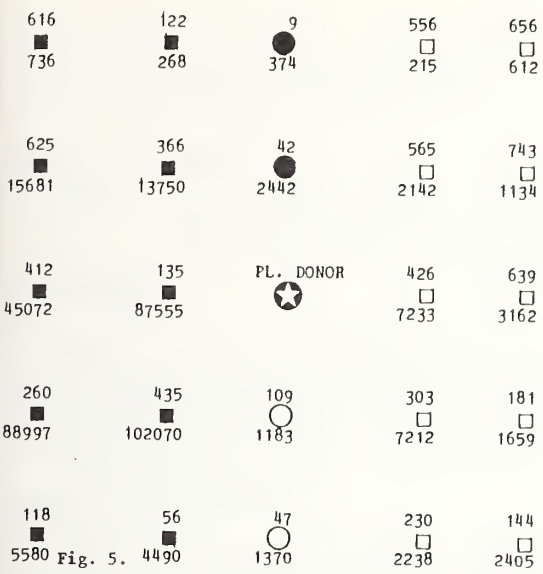


Fig. 5.

Distribution of plants and of radioactivity (d.p.m. mg d. wt.) in simulated sward of mycorrhizal Festuca (square symbols) and Plantago (circular symbols) plants, 48 hours after feeding the central Plantago donor (PL. DONOR) with $^{14}CO_2$. Closed symbols represent shaded plants open symbols fully illuminated plants. The upper figure at each plant is shoot radioactivity, the lower root radioactivity.

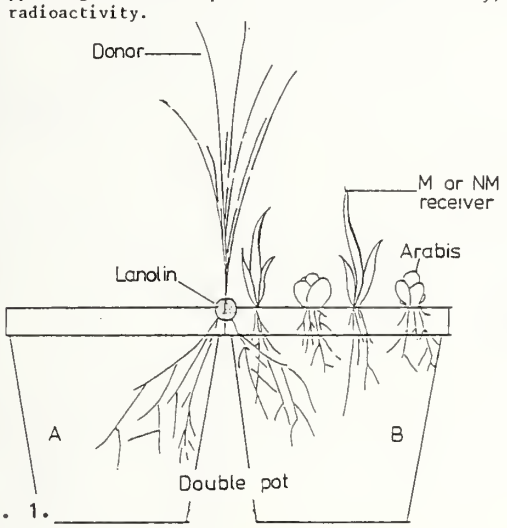


Fig. 1.

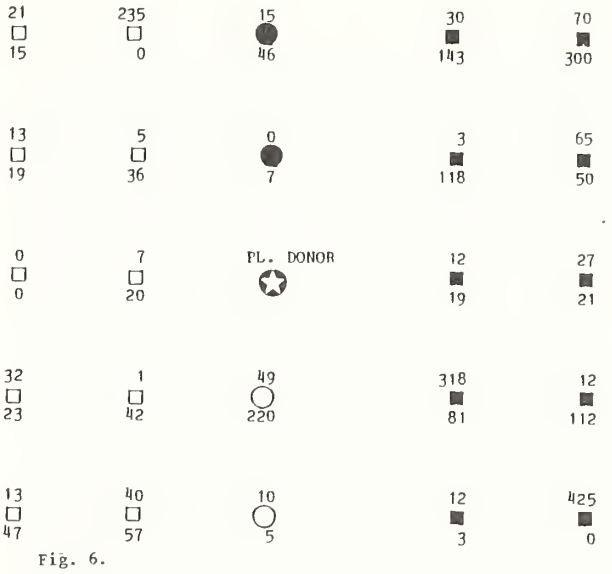


Fig. 6.

Details as in Fig. 5 but all plants non-mycorrhizal.

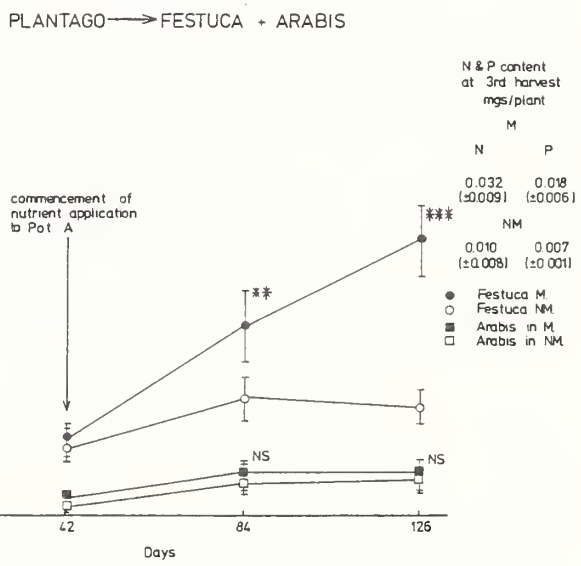


Fig. 7.

Fig. 1. Double pot system used in nutrient transfer experiment. Fig. 7. Representative example of type of growth response obtained in mycorrhizal (M) and non-mycorrhizal (NM) receivers in double pot system. In this case Plantago was the 'donor' and Festuca and Arabis were 'receivers'.

examining species rich vegetation, concluded that the capacity of plants to coexist depends on their ability to tolerate periods of low light and poor nutrient supply rather than upon their competitive ability. The transfer of resources between plants growing together in such stressed situations may be a vital factor in the dynamics of the community.

3. The ecosystem. Direct inter-plant transfer of nutrients reduces the losses due to leaching and to immobilization by the general soil microflora. Tighter nutrient cycling would lead to more effective conservation of resources and to sustained productivity.

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[illegible] COMMUNITIES.

By

G. Puppi, A. Tabacchini, W. Chiarreri and F. Carrico.

Keywords - Sand dunes. *Ammophila littoralis*. *Gigaspora*. *Glomus*, *Acaulospora*

Introduction

The presence of vesicular-arbuscular mycorrhizae and endogonaceous spores in sand dunes is known since many years. Mycorrhizal infection is often quite heavy, and the extramatrical mycelium may constitute the main factor in sand aggregation (Sutton and Sheppard, 1976). It seemed therefore of interest to examine the mycorrhizal conditions of plants growing on sand dunes and the aboundance and type of Endogonaceae in littoral areas in central Italy.

Methods and materials

The main sampling zone is located in the Presidential estate of Castelporziano, near Rome, and is formed by a series of dunes extending up to 100 m from the wash zone (for vegetational data, see Gratani *et al.*, 1983). Average pH is 6.8; N content varies between 0.8 and 1.4 ppm, and P content is about 0.02 ppm ; the highest values are recorded in the first dune, and the lowest in the second. The opposite is observed for organic matter, that varies between 0.29 and 0.33 % (Gratani, unpublished data). Moisture is between 0.3 and 5%.

Sampling were made periodically, collecting plant roots and the surrounding sand; sand samples not related to vegetation were also collected. Root and soil samples were collected also in other littoral areas, in order to observe the distribution of VAM fungi and possible differences in mycorrhizal conditions of plants. Percentage mycorrhizal infection was estimated after staining by the gridline intersect method and slide-length method (50 1 cm segments). Endogonaceous spores were recovered by wet-sieving on 2 mm, 250, 100 and 50 µ sieves. Counts were made on the entire sievings from 250, 100 and 50 µ sieves.

Results and discussion

The more relevant species in the area, *Ammophila littoralis*, is always well mycorrhized. Percentage mycorrhizal infection decreases regularly from the first to the third dune, alike the cover of the species. The same was reported by Koske and Halvorson (1981) for *Ammophila breviligulata*. The ratio of mycorrhizal to non-mycorrhizal plants on the contrary increases. Mycorrhizal incidence reaches the maximum in November. Table 1 shows data of three samplings periods, and annual mean of mycorrhizal infection and ratio of mycorrhizal to non-mycorrhizal plants.

Spore numbers (Table 2) are always low, increasing from the first to the third dune, and higher in spring and lower in winter. Spore types are: two *Gigaspora* species, and several *Glomus* species, possibly *G. macrocarpum*, *G. fasciculatum*, *G. mosseae*, *G. etunicatum*, and *G. occultum*; an *Acaulospora* was also observed. The frequency of the different types varies from site to site; relations to plant species and dune series are not observed.

Table 1. Mycorrhizal infection in *Ammophila littoralis*.

Dune	February	April	November	Annual mean	M/NM
1	28.74	35.7	57.75	30.0	0.71
2		10.64	38.6	25.6	0.82
3	21.26	2.5	31.7	20.4	0.89
mean	25.0	24.8	42.7	26.3	
M/NM	0.80	0.87	1.0		0.80

Table 2. Spore numbers/100 g d.w.

Dune	February	April	November	Annual mean
1	2.05	5.17	2.95	7.03
2		7.8	4.9	5.44
3	3.54	49.26	15.05	25.97
mean	2.9	21.5	8.9	12.8

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THE ECOLOGY OF VESICULAR-ARBUSCULAR MYCORRHIZA UNDER TWO TROPICAL SOILS

By

A. Chulan, M. Omar & M. F. Nor'Aini

Keywords -- Bungor soil, ecology, Gigaspora, phosphorus, rainfall, sand tailings

Introduction

The distribution of vesicular-arbuscular mycorrhizal (VAM) spores has been strongly associated with several factors (Saif & Khan, 1975; Saif et al., 1975; Sheikh et al., 1975; Chulan et al., 1983). The distribution of VAM spores from two different locations have been assessed in this study.

Methods and Materials

Soil samples were collected from two locations: i) a 4-year old cocoa farm under Bungor series and ii) an uncultivated wasteland of sand tailings overgrown with a weed, lalang (Imperata cylindrica). They have the following properties respectively: pH 4.7, 7.5; clay 57.0, 9.6 %; sand 38.8, 22.5 % and extractable P 5.5, 9.0 ppm. Five subsamples of soil were collected from the rhizosphere of plants from each location. The respective subsamples were bulked to constitute one sample. A total of three samples were collected from each area.

VAM spores were recovered from both soil types by the wet-sieving and decanting technique of Gerdemann & Nicholson (1963) followed by the sucrose centrifugation technique of Tommerup & Kidby (1979). Individual healthy spores were picked from the filter paper. Two hundred spores from the respective areas were used as inoculum on cocoa seedlings and lalang rhizomes respectively. The remaining spores were mounted in lactophenol for identification.

Soil samples were also collected at a three-month interval from one particular cocoa tree for isolation of VAM spores.

Results and Discussion

A higher spore count was obtained from sand tailings than Bungor soil (Table 1). The almost neutral pH of the sand tailings supported the growth of three mycorrhizal species, Acaulospora, Glomus and Gigaspora. Bungor soil type with pH as low as 4.7 favoured Gigaspora species.

One cocoa tree growing in low soil P gave a high spore count (180 spores /100g soil) compared to other trees in the vicinity. Rainfall influenced spore numbers (Fig. 1). A high rainfall resulted in a lower spore count. It is possible that with sufficient water supply, fungi rapidly grow vegetatively while a sudden drop in water supply could create an adverse situation stimulating them to produce spores. Similar trends were observed by Chulan et al. (1983).

Plate 1 shows the characteristic large circular vesicles in cocoa roots colonized by Gigaspora spores. Plate 2 shows a large ornamented spore isolated from the sand tailings.

Results from this survey indicates the dominance of Gigaspora species in Bungor soil while it only constitutes a third of the total spore population from sand tailings. In the cocoa area which received mineral fertilizer, high nutrient levels in the soil may be a contributory factor to the low spore count made. This is in contrast to observations made by Abbott & Robson (1977) who recorded higher spore counts in cultivated soils than in virgin soils.

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Soil Sample	extractable P (ppm)	soil pH (H_2O)	no of spores / 100g soil	spore types
Location I				
A_1	35.5	4.7	82	Gi sp
A_2	18.9	4.7	180	Gi sp
A_3	147.6	4.8	11	Gi sp
Location II				
B_1	9.5	7.5	332	A/Glo/Gi
B_2	9.2	8.5	287	A/Glo
B_3	8.4	7.3	182	A/Glo

Table 1: Soil characteristic of samples with number and types of VAM spores isolated

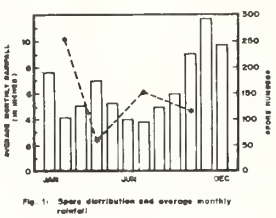

Fig. 1: Spore distribution and average monthly rainfall

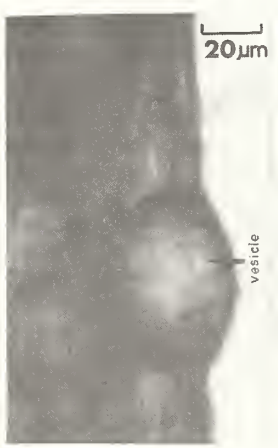

Plate 1

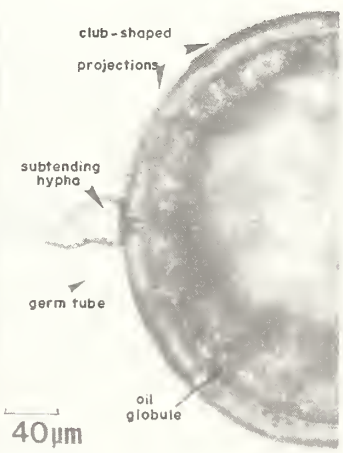

Plate 2

IRREGULAR HYPHAE PRODUCED BY SPORES OF GLOMUS VERSIFORME(EPIGAEUM) IN THE ABSENCE OF BACTERIA THAT STIMULATE GERMINATION. Kathryn Mayo and Robert E. Davis, University of Maryland, College Park, MD 20740 and USDA, Beltsville Agricultural Research Center, Beltsville, MD 20705.

Germination of surface disinfested spores was significantly increased by addition to the spores of bacteria previously isolated from non-surface disinfested spores. Hyphae emerging from spores in the presence of the bacteria were smooth, developed small vescicles, and were substantially longer and more extensively branched than those from surface disinfested spores without bacteria. The latter hyphae were characterized by meager growth, an abnormal knobbed appearance, and a lack of vescicle formation. Spore-associated bacteria capable of stimulating G. versiforme spore germination were characterized by a variety of physiological test and found to belong to several different genera, including Pseudomonas and Corynebacterium.

VA MYCORRHIZAE IN NATURAL RED MAPLE SEEDLINGS IN NEW YORK STATE

Peter Tobiessen
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Previous work (Tobiessen and Werner, 1980, Ecology 61;25) described a suppression of VAM colonization of red maple seedlings growing under red pine plantations. The uncolonized seedlings had very low levels of foliar phosphorus and did not survive under the red pine plantation at the experimental site.

To determine if the observed VAM suppression was a more generalized phenomenon, young red maple seedlings were sampled from a variety of sites including other conifer plantations and natural stands of red and white pine, hemlock, and various deciduous stands of different successional status.

The results (Table 1) show that VAM colonization in red maple seedlings was very low in plantations of red pine, white pine and Norway spruce, and in a natural stand of red pine. The density of pines in the natural white pine stand was low, and no suppression was found there. In the deciduous stands VAM colonization was high. In almost all cases, red maple seedling that lived for four or more years were colonized, even at the sites where there was suppression in young seedlings, suggesting that the VAM association is important to the survival of red maple under field conditions in this area.

Table 1. Red maple colonization by VAM according to collection site and age. Each fraction in the table represents the number of seedlings with no VAM over the total number of seedlings sampled.

	Seedling Age		
Site	1 yr	2-3 yr	4-4+ yr
Plantations			
Red Pine I	40/42	6/6	1/8
" " II	7/7	2/6	1/9
" " III	4/7	6/10	0/5
Scotch Pine	0/42	0/8	
Norway Spruce	6/6	3/3	
White Pine	4/4	2/2	2/3
Natural Stands			
Red Pine	10/17	0/1	0/1
White Pine	1/6	0/4	0/3
Hemlock	0/3	0/7	0/1
Beech-Maple	0/10		
Birch-Maple	0/7	0/4	0/1
Oak-Maple	0/2	0/19	
Old Field I	0/6	0/1	0/3
" " II	0/16	0/4	0/1
Open Road Bed	1/7		0/1

OBSERVATIONS OF VAM SPORES INHABITING WEED SEED IN NORTHEAST TEXAS SOILS

By

E. M. Arvanetes, and R. A. Taber

Keywords--Glomus, Gigaspora, weed seed

Introduction

Viable Glomus and Gigaspora spores are known to inhabit weed seed in fine, sandy-loam soils in Northeast Texas. Glomus spores inhabit 40% of the retrieved seed whereas Gigaspora spores inhabit 4% of the retrieved seed. Reports indicate this relationship is not unique to Northeast Texas. Other study sites include Northwest and Southeast Texas, Thailand, and the Philippines. This report concerns (1) observations of the hyphae and spores within the seeds and (2) characteristics of spores extracted from established pot cultures. The goal of these studies is to eventually assess the effectiveness of these fungi for enhanced plant growth.

Methods and Materials

Weed seeds were retrieved from the soil by a modified sieving technique (Gerdemann and Nicholson, 1963) using US standard sieves 425µ and 250µ. Seeds were cracked and examined for presence or absence of spores under the stereomicroscope.

Greenhouse pot cultures were established on sudangrass grown in an autoclaved fine, sandy-loam soil collected from the study site. Approximately 20-30% of the root length of sudangrass roots was colonized 90 days after soil infestation.

Spores were extracted from the soil by wet sieving and flotation in 40% sucrose. Glomus and Gigaspora spores were separated and examined.

Results and Discussion

1. Only Glomus and Gigaspora spores have been observed in weed seed.

2. Only on one occasion have spores of both genera been observed in the same seed.

3. The majority of the Glomus spores in seeds (Fig. 1) are borne in groups of up to 20 chlamydospores, measure up to 107µm across, are hyaline to light yellow, always smooth, globose, have no hyphal envelop, a single wall (<3µm) and have a cylindrical subtending hyphae up to 4µm at the point of attachment.

4. The common Gigaspora azyspores agree with descriptions of G. margarita. Another species has been tentatively identified as G. gregaria. No extramatrical vesicles have been observed in weed seed.

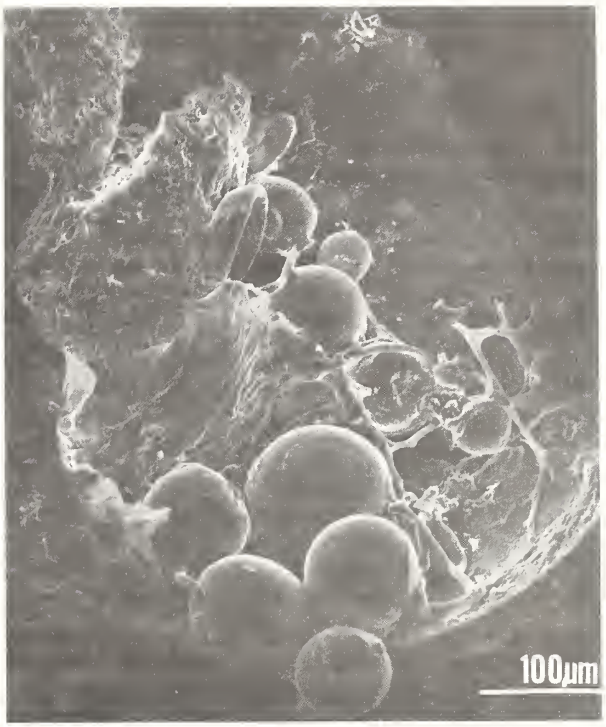

Figure 1. Glomus spores inhabiting weed seed.

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INTERACTIONS BETWEEN VA MYCORRHIZA AND PHOSPHATE SOLUBILIZING BACTERIA

By

W. Krone, B. Bichler, E. Viebrock, and A.M. Moawad

Keywords--VA mycorrhiza, phosphate bacteria, phosphorus, tropical and subtropical plants, soil pH, soil temperature

Introduction

Phosphorus in tropical and subtropical soils is mostly fixed and is not available to plants. Improved growth in the presence of VA mycorrhiza has been demonstrated many times and is greatest in soils of low fertility containing little available phosphate (Mosse 1973). Cooper (1959) and Kundu and Gaur (1980) have reported on a promoting effect of phosphate solubilizing bacteria on plant growth. The aim of these investigations was to study the interactions between the mycorrhizal symbiosis and bacterial fertilizers in phosphorus nutrition of tropical and subtropical plants.

Materials and Methods

Experiments were conducted in a glasshouse with *Capsicum annuum*, *Guizotia abyssinica*, *Solanum melongena*, *Sorghum bicolor*, *Stylosanthes guianensis*, *Tagetes minuta* and *Trifolium alexandrinum*. The interactions were tested between the three mycorrhizal fungi *Glomus macrocarpum* (M_1), white reticulate (M_2) and *Acaulospora spinosa* (M_3) and the four bacterial species *Bacillus megaterium* var. *phosphaticum* (B_1), *Pseudomonas fluorescens* (B_2), *Pseudomonas stukeri* (B_3) and *Citrobacter freundii* (B_4). The plants were inoculated with roots of *Eupatorium odoratum* mother plants containing mycorrhizal fungus, and transplanted into one-kg plastic pots filled with steamed acidic soil/sand mixture with low phosphate availability. Ten ml bacterial suspension of 4.5×10^8 cell/ml were added into the rhizosphere per pot. The soil was fertilized with N, K and Mg. Phosphorus was applied as $Ca_5(PO_4)_3OH$ or $FePO_4$. To examine the effect of soil pH, in some experiments the soil was treated with $CaCO_3$ to give pH calues of 5.5, 6.5 and 7.5. In other experiments soil temperatures were tested of 20 °, 25 °, 30 ° and 35 ° C maintained in water baths regulated by thermostats. After harvesting, representative root samples were used for the microscopic examination of mycorrhiza. The dried plant shoots were analysed for phosphate. Number of living bacteria was estimated in the soil.

Results and Discussion

The mycorrhizal fungi showed different efficiencies in improving plant growth; *G. macrocarpum* > white reticulate > *A. spinosa*. The efficiency of *G. macrocarpum* was always superior at higher pH values, whereas white reticulate, *A. spinosa* and all species of bacteria were not influenced by soil pH. In many cases inoculation with bacteria promoted plant growth and P uptake. However, the efficiency of bacteria was always lower than of mycorrhizae. No correlation was found between the number of living bacteria and plant growth. Often the combined inoculation with bacteria and fungi enhanced plant growth and P uptake (Fig. 1). Barea et al. (1975) and Azcon et al. (1978) have also reported on positive influence of combined

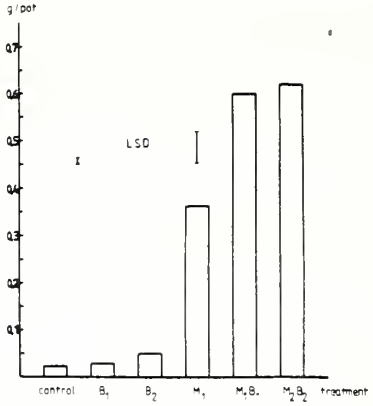

Fig. 1. Dry weight of *Capsicum annuum* inoculated with bacteria (B_1, B_2) or/and fungus (M_1) fertilized with $FePO_4$ at soil pH 6.5

inoculation with VA mycorrhizal fungi and phosphate solubilizing bacteria on plant growth and P uptake of maize, lavender, lucerne and tomato. In some cases inoculation with bacteria or mycorrhiza alone had no influence on plant growth, but the combined inoculation enhanced plant growth significantly. In several cases plant growth was improved by the combined inoculation over that of plants inoculated only with mycorrhiza, although bacteria alone could not increase dry matter content of plants. Furthermore, the combined inoculation showed in some pH treatments with *C. annuum* and *S. melongena* fertilized with $Ca_5(PO_4)_3OH$ a synergistic effect on plant growth. Highest efficiency of combined inoculation was found at higher soil temperatures. These results demonstrate that the combined inoculation with VA mycorrhiza and P-solubilizing bacteria can have very favourable effects if attention is paid to other growth factors such as soil temperature and pH.

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THE SPATIAL DISTRIBUTION OF INFECTION IN TRIFOLIUM SUBTERRANEUM

by

S. E. Smith and N. A. Walker

Keywords - entry point distribution, Trifolium, infection unit size

Introduction

The spread of mycorrhizal infection in the roots of young plants has usually been studied by collecting data on the fraction of the root infected, together with, in some studies[1], the number of entry points per plant. The attempt to model such data involves a number of assumptions - e.g. that infection takes place at random on the root system - that can be checked best by having more complete data on the positions of infection units on the root system. Maps of the infection of a root are tedious to prepare, but recently the mapping of infections in leek roots[2] has allowed interesting conclusions to be drawn.

We have prepared maps both of infections and of entry points in clover plants, with the aim of testing assumptions or predictions of our model by the use of suitable statistical tests.

Methods and Materials

We have used Trifolium subterraneum cv. Mt. Barker, grown from 5 days in soil containing a mixture of indigenous mycorrhizal fungi including Glomus mosseae. Details have been published elsewhere[3]. The fungal propagules were mixed randomly and uniformly throughout the soil.

Results and Discussion

1. Maps which include both the entry points and the infection units allow us to study the relationship between the number of entry points and the number of freely-advancing infection fronts in the root system. This is equivalent to finding the number of infection units which overlap. Our postulate[1] was that for a root infected with U units, with an infected fraction l, there would be, as a result of overlapping, not 2U but $2U(1-l)$ advancing fronts.

The mapping of a clover plant at 16 days gave the following results:

Type of Root	U	l	$2U(1-l)$	Observed #
Main	17	0.5	17	12
Lateral	97	0.24	147	144

We conclude that at moderate densities of infection the term $(1-l$ may give a satisfactory allowance for overlap in infection units.

2. Some models for mycorrhizal infection spread have tacitly assumed that infection units were all of a standard size, so that l could be found from U and the root length L[4].

Mapping allows us to inspect the distribution of lengths of those infection units which do not overlap; the lengths of those which do overlap are not at present accessible.

We find a roughly rectangular distribution of lengths from 0 to 1.5 mm, with a long "tail" to 8 mm. Whatever allowance is made for bias caused by the omission of the overlapping units, there is clearly a wide distribution of sizes.

3. Our equations[1], if taken with parameter A constant in time, suggest that older parts of a root should have a higher density of infection units. We have combined data for the position of entry points on two main roots (n=32) and compared the cumulative distribution with simple models, by means of the Kolmogorov Test. At a significance of 5%, neither a uniform distribution nor one resulting from constant A will fit the data. Inspection shows that this is because the basal 2 cm (0.2 of the length) is not infected. Since laterals springing from this region are infected, this seems to result from lack of infectibility of this part of the root.
The cumulative distribution of position fits a uniform distribution if the basal 0.2 of the root length is omitted: but even with this allowance it still does not fit the distribution resulting from constant A.

We conclude that (i) the basal 2 cm of the main root is not infectible in these experiments, and (ii) that the distribution of entry points favours a fall in A with time in main roots (see also [5]).

4. Lateral roots have widely varying lengths and ages and few entry points per root, so that combining data is less easy. We have, for the laterals, taken the latest entry points, associated with the youngest infections, and asked whether they are equally distributed between the basal (older) and apical (younger) halves of their root. For 42 such entry points associated with units shorter than 1.8 mm, there were 18 in the basal half and 24 in the apical half - which is well within the expectation for a proportion of 1:1.

We conclude that at 16 days the older and younger halves of lateral roots are equally infected.

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DEVELOPMENT OF VA MYCORRHIZAL INFECTION IN CRUCIFERAE AND TRIFOLIUM SUBTERRANEUM

By I.C. Tommerup

Keywords--Glomus caledonium, Brassica, Raphanus, Lobularia, specificity, phosphorus, spore germination

Introduction

Although members of the Cruciferae have often been found to be non-mycorrhizal, a small amount of colonization by VAM fungi has been reported in a few species (Hirrel et al., 1978; Ocampo et al., 1980; Ross & Harper, 1973) indicating resistance is non-specific. Little is known of the processes which result in either success or failure of colonization. To provide this type of information the rate and extent of each developmental phase leading to colonization in spring rape and subterranean clover by Glomus caledonium have been compared. In addition the development of VAM fungi in Cruciferae growing as weeds in low phosphate soils with natural inoculum has been examined.

Methods

Morphological and cytological aspects of the interactions between spores of G. caledonium and Brassica napus or Trifolium subterraneum were examined. Plants were grown in steamed soil with phosphorus added to give 70% of maximum yield in a controlled environment. Blocks of soil containing young, flowering, crucifer plants were removed from the field, washed free of soil and only attached roots were cleared and stained to show VAM fungi.

Results and Discussion

For 70% of maximum yield, B. napus required less phosphorus than T. subterraneum. B. napus reduced the rate of each developmental stage from spore germination to colony initiation compared to T. subterraneum (Table 1). Maximum values of germination, hyphal extension, and appressorial formation and adhesion, were similar between hosts, but in B. napus fewer appressoria produced penetration pegs and some pegs failed to initiate colonies. In B. napus only a third of the colonies possessed arbuscules. The rate of death of epidermal and cortical cells was similar between species and did not explain the reduced colonization of B. napus Chemical rather than physical factors associated with B. napus may be involved in reducing the rate of development of each growth stage of the fungi.

Colonies of VAM fungi were found in all crucifer species examined (Table 2). Morphological characteristics (Abbott, 1982) indicate many fungi are compatible. Development of arbuscules in several species of crucifers indicates that functional mycorrhizae can form in this group and any resistance is non-specific.

Table 2. Natural infection in Cruciferae

Plant	Fungus	Arbuscules[a]	Vesicles[a]	Spores[ab]
Brassica napus	Glomus fasciculatum	+	+	-
B. juncea	Glomus sp.	-	+	-
Raphanus raphanistrum	Glomus monosporum	-	+	-
	Glomus tenue	+	-	-
	Glomus sp.	+	+	+
	Gigaspora calospora	+	-	+
Lobularia maratima	Glomus sp.	+	+	-

[a]+, presence, -, absence; [b]attached to extraradicle hyphae continuous with entry structure

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Table 1. Reduction in the rate of development of the early stages of the interaction between Glomus caledonium and roots of Brassica napus compared with Trifolium subterraneum.

Plant	Time after planting (weeks)	Spore germination (%)	Hyphal length per spore (mm)	No. of appressoria per plant	No. of penetration pegs/ plant	Size p° colonies (No.infected cells per plant)	No. arbuscules per plant p° colonies	No. vesicles per plant p° colonies
B. napus	2	9±4	0.1±0.02	0	0	0	0	0
	3	4±2	0.2±0.03	0	0	0	0	0
	4	60±31	28±4	7±3	4±2	1±0.1	0	0
	6	100	51±4	12±2	6±2	1±0.2	1	0
	8	98±1	44±8	10±4	4±0.3	2±0.3	1	0
T. subterraneum	2	93±2	34±4	3±0.3	2±0.5	2±0.5	0.5	0
	3	100	61±4	11±2	11±2	47±7	21±4	2±1
	4	98±1	58±6	10±3	10±3	123±6	86±5	6±3

Values are means with S.E.M.; p°: colonies are primary colonies only

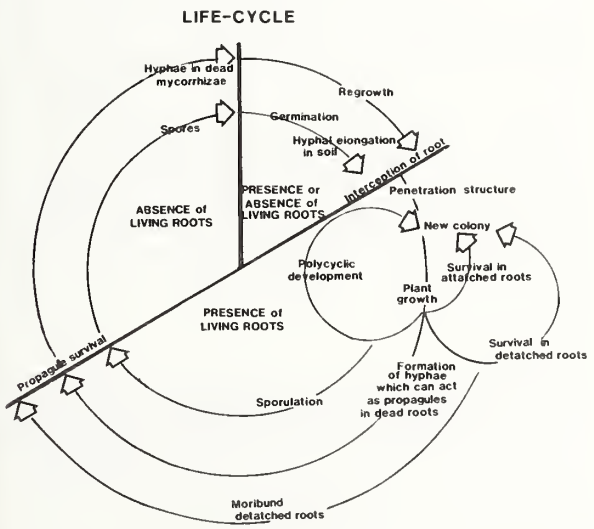
POPULATION BIOLOGY OF VA MYCORRHIZAL FUNGI: PROPAGULE BEHAVIOUR

By I.C. Tommerup

Keywords--Glomus caledonium, Acaulospora laevis, Gigaspora calospora, dormancy, survival, spore germination, life-cycle ecology

Introduction

Models of population growth of VAM fungi need to take into account the pattern of propagule production and establishment determined by events of the life cycle of the fungus. Many of these events occur in association with a host plant especially the stages leading to the production of new propagules. The models must include the behaviour of each fungus, the host plant and the interaction between them, whether the fungi already inhabit the soil or are introduced as inoculum. In many environments the presence of living roots in the surface soil is seasonal or unpredictable because of the climate, or agricultural practices. These environments can support the growth of perennial and annual plant forms. Critical phases in the development or maintenance of populations include those of persistence of dormant or quiescent propagules, their subsequent germination, growth in the soil, survival of the germinated structure until it establishes a colony in a host, and the production of new propagules prior to root death. The life-cycle interacts with the geological, meterological and biological aspects of the environment and agricultural practices resulting in success or failure to persist. The progeny of VAM fungi include the propagules, extent of colony development in roots and extraradicle hyphae. Propagules comprise spores, hyphae in dry, dead roots, and hyphae (± vesicles) associated with attached or detached roots. Major roles of propagules are perennation, multiplication, display of genetic variation and dispersal.

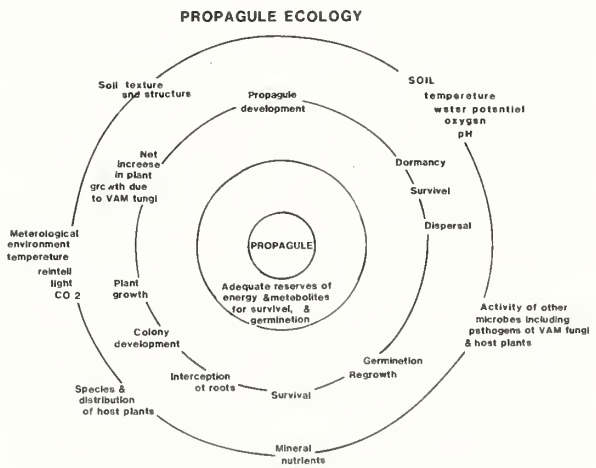


Methods

Components of the life history of several VAM fungi have been examined and the information integrated to provide an explanation of the behaviour of propagules in natural field populations. The isolates were derived from one place and subsequently grown under identical conditions. Differences in behaviour may therefore be attributed to innate diversity in function among the fungi.

Results and Discussion

Several publications contain the detailed results. Natural or introduced inoculum varies in age and chemistry. Time, spore physiology and storage conditions influence survival. Innately dormant spores fail to germinate when exposed to physical and chemical conditions which are conducive to germination of apparently identical, but non-dormant spores of the same species. The minimum dormancy period varies among fungi. The change to a quiescent state (non-dormant) is a function of time, temperature and soil water potential, but is not influenced by plants. Quiescent spores do not germinate only because the physical or chemical environment is unsuitable. Spore germination is suppressed in the soil in which they form and contributes to propagule survival. Suppression is relieved by new crop growh. The time lapsing before the onset of germination varies with the species, soil temperature and moisture. The rate of hyphal development may be reduced but the maximum length may be unaltered. Such delays can modify the spatial relationship between propagules and roots, and therefore at a given time the extent of colonization of roots. Elimination of the lag by germinating spores prior to sowing increases rates of colony initiation. In the absence of plants, germinated spores can retain their capacity to act as inoculum for a few months in natural soils. Hyphal propagules in dry dead roots have most of the survival characteristics of spores. Germinated propagules must intercept a root, initiate a colony and develop new propagules before colony death to ensure the continuity of the genotype. Propagule production can be described as the amount and duration per colony, plant or community. Although the particular life history reflects the habitat from which the fungi were isolated, many of the characteristics occur in the same species from quite different habitats. Functional diversity associated with persistence may dominate the biology and hence the population development of many VAM fungi.



INHIBITION OF ROOT PRODUCTION AS THE MECHANISM OF PATHOGENICITY OF GLOMUS MACROCARPUM TO TOBACCO

By

Keith Jones and J. W. Hendrix

Introduction

Tobacco yields in Kentucky have been reduced by a previously undescribed disease. Tobacco extension workers estimate that statewide loss to this stunt syndrome in 1983 was 5.7% (about $27 million in 1983). The symptoms of stunt appear within a month of planting. The affected plants do not grow or grow only slowly, but seldom die and mature (flower) much later. Roots of stunted plants appear similar to those of healthy plants except that the roots are poorly developed.

Modjo (1983) eliminated Thielaviopsis basicola, pratylenchus spp., other nematode, and Fusarium spp. as potential pathogens in tobacco stunt. Modjo also showed that the cause of the stunting was able to pass through a 864 um sieve but not through a 100 um sieve, and that stunting of plants in the greenhouse was correlated to the presence of Glomus macrocarpum.

This study was undertaken to investigate further the role and mechanism of G. macrocarpum in the stunt syndrome of Burley tobacco in Kentucky.

Effect of Glomus macrocarpum on Root Development

Procedure:

Experiments were conducted in 165 ml growth tubes. The soil was shredded, steamed in bulk and stored. Before use, the soil was fertilized with tobacco fertilizer at rates used in the field, and then microwaved.

Soil infested naturally with tobacco stunt pathogen was collected from the site of the field experiment. Spores of G. macrocarpum were isolated from single spore culture maintained on alfalfa. The cultures were isolated from soil from the site of the field experiment. Spores harvested by wet sieving and centrifugation in 65% sucrose solution were surface disinfested by immersion in 0.5% sodium hypochlorite solution, followed by washing three times with sterile distilled water.

Inoculated plants were grown for 50 days in the greenhouse after which they were harvested and assessed for growth and mycorrhizal infection. There were 14 plants in each treatment.

The experiment was repeated two other times with similar results.

Field Experiments

Field experiments were conducted at the Perkins Farm (Scott County, Kentucky) where the 1979 tobacco crop was a near failure. A three year rotation experiment was initiated in 1980. The treatments were continuous tobacco; tobacco 1980, red clover/fescue mix 1981; and red clover/fescue mix in 1980 and 1981. All blocks were planted to tobacco in 1982.

Fumigant was applied about 10 days prior to transplanting. The fumigant used was methyl bromide: chloropicrin (2:1 w/v) at a rate of 435.8±13.6 Kg per Ha.

30 and 70 days after planting data was taken from 5 individual plants in each plot. Soil cores (2.5 cm diameter) were taken from near the base of each plant, root pieces and endogonaceous spores were collected from these soil samples by wet seiving and decanting. Root length and % colonization by endogonaceous fungi were estimated using a root/gridline intercept technique after clearing with 10% KOH and staining with Trypan blue in Lactophenol.

Each treatment was replicated three times.

Greenhouse Results

G. macrocarpum and stunting soil resulted in a drastic decrease in root mass and length, and a slight decrease in shoot growth when compared to control plants. Benomyl significantly reduced the inhibitory effects of G. macrocarpum on root production.

Field Results

Rotation for two years out of tobacco resulted in an early increase in root production, a decrease in % root colonization by endogonaceous fungi and a decrease in populations of spores of G. macrocarpum as compared to continuous tobacco. In continuous tobacco, fumigation resulted in a decrease in % root colonization, a decrease in spore populations of G. macrocarpum, an increase in the root length produced per plant, and an increase in yield. With one year out of tobacco similar fumigation effects were less marked. There were no significantly significant fumigation effects with two years out of tobacco.

Conclusions

G. macrocarpum decreases root production by tobacco. Under greenhouse conditions this may not result in much reduction in shoot growth.

In the field the reduced root production may result in stunting of above ground plant parts. The field data is consistent with this hypothesis.

Reference

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INCIDENCE AND SURVIVAL OF VA MYCORRHIZAL FUNGI IN PADDY RICE CULTURE.

BY

O. Nopamornbodi, N.C. Schenck and Y. Vasuvat

Keywords--VAM Fungi, spore survival, Paddy rice.

Introduction

In Thailand, rice is the most important crop with about 37 % of the agriculture land in rice. There are numerous reports on the incidence of vesicular arbuscular mycorrhizal VAM fungi on the roots of plants but only one study on paddy land rice has been reported by Mnbaga. This study was undertaken to determine the number of VAM spores present in paddy soil in the north, northeast and central part of Thailand. This represents the first study in which the incidence of VAM fungi was determined before and after flooding in rice paddies.

Materials and Methods

In each of 54 paddy rice fields sampled, soil samples were randomly collected at 0-15 cm depth and composited. One hundred grams of soil from each composite sample were wet seived. Spores and sporocarps on 450,250 and 63 µm seives were mounted in lactophenol on glass slides for identification by using keys of trappe and Schenck et al. Root samples colected were stained as described by Phillips and Hayman and the mycorrhizal colonization percentage was determined by the root slide technique.

Results and Discussion

Spores of VAM fungi were present in all areas sampled. Spores less than 250 µm in diameter were found in 46 of 54 soil samples collected. Spore numbers in the soil varied from zero to 27,000 spores in 100 g of soil. Spores of mycorrhizal fungi were found in soils ranging from pH 4.4 to 7.7, in soil having available phosphorus levels of 3.0 to 39 ppm and in soil texture ranging from sandy loam to clay. There was no apparent relation ship between the spore number of VAM fungi and either soil texture, soil pH or available soil phosphorus.

The monthly occurence of spore numbers for VAM fungi from paddy rice fields is shown in Fig 1. Spores numbers increased with each sample date after March 1983 and reached their maximum in September 1983 but during the booting stage of rice only a few spores has cytoplasmic contents and appeared viable. The number of spores decreased in January 1984 and started to increase again in the following month.

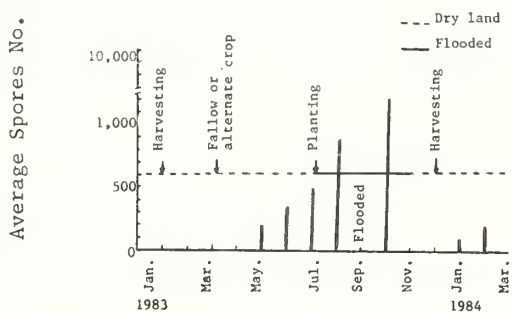
Acaulospora morrowae was found commonly in most paddy fields. Gigaspora gregaria, Acaulospora laevis, Gigaspora heterogama, Gigaspora margarita and Gigaspora gigantea were found commonly in Inceptisols soils. Sclerocystis spp. and Entrophospora spp. were found only in a few paddy fields.

Several weeds in a fallow rice paddy 60 days were sampled for the evidence of hyphae of VAM fungi in their roots. These weeds included several species of Gramineae, Leguminaceae, Euphorbiaceae. Root colonization ranged from 3 % to 75 % with Heliotropium indicum having the highest percent root colonization of 75 % and Chrozophora rottleri having the lowest percent root colonization of 3 % .

The incidence of spores of VAM fungi changed with time. Spore numbers were relatively high in rice paddy soil before flooding and increased tremendously after flooding but spores were largely devoid of contents at this stage. Few spores could be recovered at 60 days after harvest but shortly thereafter (60 days) roots of indigeneous weeds in the paddy were well colonized by mycorrhizal fungi. The source of this root colonization was not determined but it is unlikely that viable spores in the upper 15 cm of soil provided the inoculum source. Further studies on the nature of the survival propagules in flooded rice paddies will be initiated.

Although species in four genera of the family Endogonaceae were observed, the predominant species were in the genera Acaulospora and Gigaspora. Quantitative and qualitative dif - ferences in VAM fungi could not be attributed to difference in physical or chemical characteristics of the soil since only slight differences occured in this regard. The extent to which spores of VAM fungi reflect inoculum potential in rice paddies still unknown.

Fig 1. Average spore numbers of VA mycorrhizal fungi in paddy rice fields in 1983 - 1984.



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THE NEMATODE-TRAPPING EFFECTS OF MYCORRHIZA IN TROPICAL SOILS

By

Eng. Dr. Francisco R. Tamas

Keywords--Fusarium, Trichoderma, Gliocladium, Basidiomycetes, Biorgan Soil Reconstructor, nematode traps.

Introduction

Field tests on rice plantation in the Cibao region of Santo Domingo proved that certain mycorrhiza act as efficient nematode traps. This effect was enhanced by systematic appliction of "Biorgan" soil reconstructor, rich in nematode-trapping fungi. These mycorrhizas use nematodes as a supplemental food.

Methods and Materials

Our principal method was the use of "Biorgan" soil reconstructor, because it offers a rich substrate for mycorrhiza spore germination. "Biorgan" is a specific mycorrhiza producer and contains 60 of the most important microelements, plus auxins, enzymes, proteins and other micronutrients which act synergistically with the germination of the spores of mycorrhizal fungi.

Results and Discussion

Soils low in organic matter have been found to contain low levels of predacious fungi. As a result, the predatory nematodes are free to create serious problems, unless they can be controlled through applications of expensive chemical nematocides.

Significant reductions in the concentrations of rice root nematodes in Dominican rice fields were observed as a consequence of the activity of predacious mycorrhizal fung such as Fusarium, Trichoderma, Gliocladium and Basidiomycetes following the incorporation of "Biorgan" soil reconstructor into the soil.

Microscopic investigations showed that mycelium of the fungi contain short branches, which curl around and unite with adjacent branches or with the parent hyphae to form three-dimensional networks. These fungal networks are strongly adhesive; when a nematode comes in contact with such a network, it is held fast by powerful adhesive forces. For a time the nematode struggles violently, but after a short period its activity diminishes and it perishes. At that time a fine mycelial process from the mycorrhizal fungal network penetrates the trapped nematode and swells within it into a bulbous structure from which trophic hyphae spread to all parts of the nematode.

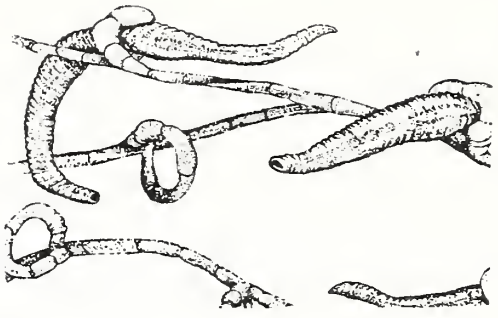

Figure 1. Nematode-trapping fungi shown here snaring two rice nematode larvae.

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PHYSIOLOGY

SURVIVAL OF *Phytophthora cinnamomi* ZOOSPORES ON NON-MYCORRHIZAL AND ECTOMYCORRHIZAL ROOTS OF JARRAH

By

N. Malajczuk and C.L. Sanfelieu

Keywords--*Eucalyptus marginata*, *Phytophthora cinnamomi*, zoospore infection

Introduction

Ectomycorrhizas have been implicated in limiting infection by soil-borne pathogens, particularly *Phytophthora cinnamomi* (Marx, 1982). In the jarrah (*Eucalyptus marginata*) forest of south-western Australia, *P. cinnamomi* is causing widespread destruction of the trees and associated understorey. This study was initiated to examine the role (if any) of natural jarrah ectomycorrhizas in deterring *P. cinnamomi* infection of fine roots.

In maturing jarrah forest, formation of ectomycorrhizas is influenced by soil stratum. Ectomycorrhizas initiated by the ascomycete, *Cenococcum geophilum* (black type) are predominantly found in mineral soils. Ectomycorrhizas found in the litter are usually initiated by basidiomycetes (white type).

Methods and Materials

Jarrah seedlings were grown in soil or litter in root boxes with one side made of clear plastic. After twelve months' growth, abundant black and white type ectomycorrhizas were formed in soil and litter. Point source inoculation of the different types of ectomycorrhizas and non-mycorrhizal roots was carried out with a *P. cinnamomi* zoospore suspension. Observation of the behaviour of zoospores on the root surfaces was carried out at periodic samplings using light microscopy and a scanning electron microscope. Roots were also selected at these time intervals and plated onto $P_{10}VP$ + hymexazol agar (Malajczuk *et al.* 1983), to estimate survival and infectivity of zoospores.

Results and Discussion

Point inoculated non-mycorrhizal and *C. geophilum* ectomycorrhizal roots (black type) of seedlings raised in soil showed accumulation and germination of zoospores on the root surface (Figure 1). The pathogen was recovered at regular intervals from both root types after 55 days' incubation (Figure 2).

Recovery of *P. cinnamomi* from non-mycorrhizal roots of seedlings growing in the litter was markedly less than in soil, and the pathogen could not be isolated from these roots after 5 days' incubation. There was no recovery of the pathogen from the basidiomycete ectomycorrhizas even though zoospores were seen to germinate on the root surface at 24 hrs. incubation (Figure 3). Many of the zoospores were colonized by a range of morphologically distinct bacteria and actinomycetes (Figure 4).

These results indicate the importance of forest litter in stimulating growth of antagonistic micro-organisms in the rhizosphere and encouraging specific ectomycorrhizas in reducing *P. cinnamomi* survival and infection.

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Figure 1. Accumulation of zoospores on non-mycorrhizal root.

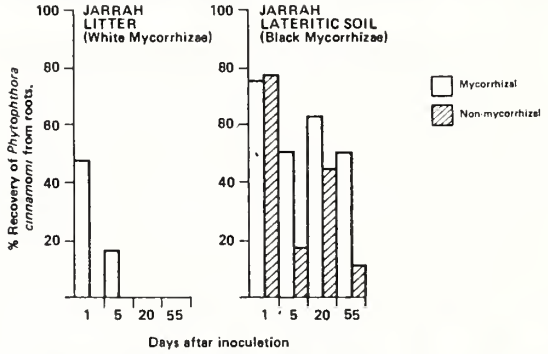

Figure 2. Recovery of *P. cinnamomi* from mycorrhizal and non-mycorrhizal roots of jarrah.

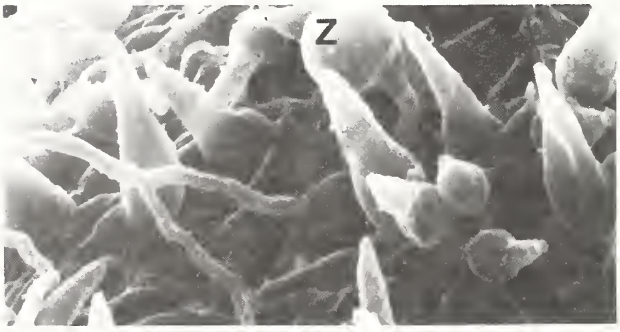

Figure 3. Germination but lack of penetration of jarrah mycorrhizal root by a zoospore (Z).

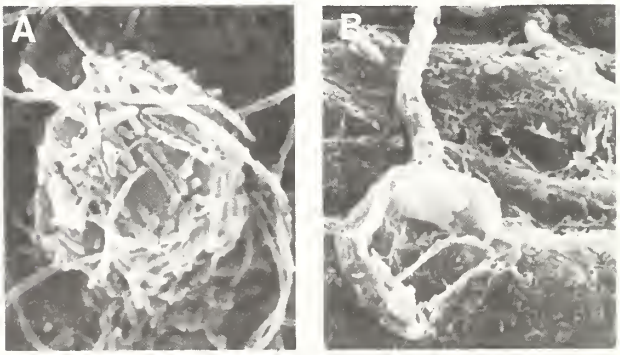

Figure 4. Bacteria accumulating on (A), and associated with a lysed (B), zoospore.

INFLUENCES OF BORON ON ECTOMYCORRHIZAL COLONIZATION AND GROWTH OF SHORTLEAF PINE

By

R.J. Mitchell, A. Atalay, G.S. Cox, H.E. Garrett, and R.K. Dixon

Keywords--Pinus echinata, Pisolithus tinctorius, container-grown

Introduction

Even though boron is an essential element in the nutrition of higher plants, the few reports on boron's role in forest tree growth have concerned deficiencies and their amelioration (Stone and Will 1965, Stone et al. 1980). Little is known about the growth and development of forest tree seedlings supplied with boron in excess of minimum requirements.

Although essential for higher plants, boron is apparently not required by fungi (Stiles 1958). Nevertheless, endomycorrhizal infection of clover and alfalfa are increased by boron (Lambert et al. 1980). The influence of boron on ectomycorrhizal infection, however, has apparently not been investigated.

The objectives of this study were twofold: first, to determine the effects of boron fertilization on mycorrhizal infection of container-grown shortleaf pine (Pinus echinata Mill.) seedlings; and secondly, to evaluate the effects of B on mycorrhizal inoculation on the growth of the seedlings.

Methods amd Materials

A sterilized sandy loam soil to which sterilized peat vermiculite (1-10v/v) or peat vermiculite Pisolithus tinctorius (Persh) Coker Couch inoculum had been added, was used as the growth medium; 150g of this medium was placed in each cavity of four cavity bookplanters. Five half-sib shortleaf pine seeds were planted in each cavity; after established, the seedlings were thinned to one per cavity.

A modified Hoaglands solution (MHS) was applied to the growth medium (20 ml/cavity) twice weekly. The fertilizer control treatment received only the MHS, while the foliar fertilized seedlings received MHS applied to the soil and 25 ppm borate solution applied as a foliar mist until the seedlings were thoroughly covered (approximately 10 ml/seedling). The soil fertilized seedlings received the MHS with 25 ppm borate added to the formulation. The foliar + soil fertilized group received the MHS + 25 ppm borate and 25 ppm borate foliar mist.

The seedlings were harvested after 16 weeks of growth. Height, diameter, foliage weight, stem weight, number of primary lateral roots, primary laterals with mycorrhizal structures, and the number of infection points per infected lateral root were determined.

Results and Discussion

Fertilization with 25 ppm borate applied as a mist to the foliage, or as a soil drench, significantly promoted the colonization of ectomycorrhizae on container-grown shortleaf pine seedlings by 135% and 121%, respectively. The increase in ectomycorrhizal infection reported in this study is consistent with the findings by Lambert et al. (1980) who demonstrated that B fertilization increased endomycorrhizal colonization of clover and alfalfa. Boron fertilization significantly affected both primary lateral infeciton percentage and the number of infection points per infected lateral. The degree of colonization was so intense in the foliar fertilized and soil fertilized seedlings that many areas of the lateral roots were completely covered with mycorrhizae. Colonization in the foliar + soil and the control seedlings was characterized by single infection points along the lateral roots. The lack of response of mycorrhizal colonization to foliar + soil fertilization compared to that observed in foliar or soil fertilization treatments may suggest that foliar + soil fertilized seedlings were exposed to supra-optimal levels of B.

Foliar fertilization with 25 ppm borate or soil application of 25 ppm borate significantly increased stem diameter, stem weight, and foliage weight, but did not increase height or root weight. The increased growth of seedlings receiving these treatments was paralleled by an increase in ectomycorrhizal colonization. Boron fertilization, however, had essentially no effect on any aspect of growth other than height for the noninoculated treatments. Increasing the level of B from 0.3 ppm borate to 25 ppm borate apparently has a great effect on the fungus-host symbiosis than on the host alone. Subtle differences in growth of inoculated seedlings between the soil fertilized and foliar fertilized seedlings were observed. Foliar fertilized inoculated seedlings had an increase of 17 percent in foliage dry weight and 33 percent increase in root dry weight as compared with the inoculated control seedlings. Soil fertilization increased foliage dry weight 33 percent, while increasing root weight by only 17 percent. The difference between the amount of foliage vs. root mass produced may affect plant growth responses following outplanting.

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BORON AND ECTOMYCORRHIZAL INFLUENCES ON IAA AND IAA OXIDASE ACTIVITY

By

R.J. Mitchell, A. Atalay, G.S. Cox and H.E. Garrett

Keywords--Shortleaf pine, Pisolithus tinctorius, Indole-3-acetic acid, Indole-3-acetic acid oxidase, Peroxidase

Introduction

Lambert et al. (1980) reported that boron (B) fertilization increased endomycorrhizal infection, and increased ectomycorrhizal infection has also been reported to be associated with B fertilization (Mitchell et al. 1984a). The manner in which B stimulates infection by mycorrhizal fungi is unknown; however, Lambert et al. (1980) suggested that B may increase infection by altering Indole-3-acetic acid (IAA) levels and IAA oxidase activity.

The hypothesis that B fertilization increases ectomycorrhizal colonization of shortleaf pine roots by decreasing IAA oxidase activity, resulting in increased IAA levels was tested. Levels of IAA and enzyme activity of enzymes involved in the oxidation of IAA as affected by mycorrhizal inoculation were also investigated.

Materials and Methods

Materials and methods described by Mitchell et al. (1984a) were also used in this study In addition indole-3-acetic acid levels were quantified by methods described by Mitchell et al. (1984b). Peroxidase activity and IAA oxidase activity were assayed by methods of Karr and Mirshura (1976) and Bohnsack and Albert (1977) respectively.

Results and Discussion

The Influence of B Fertilization on IAA Levels

Boron fertilization significantly reduced IAA levels in inoculated shortleaf pine seedlings; however, no significant differences in IAA concentrations were observed in noninoculated plants. A similar pattern of reduced IAA oxidase activity may suggest that B is affecting the symbiont, or the host-symbiont interaction to a greater degree than the host alone. These data do not substantiate the proposed hypothesis that B fertilization increases ectomycorrhizal infection by decreasing IAA oxidase activity and increasing IAA levels -- thus creating a greater flux of carbohydrates and increasing the ability of mycorrhizal fungi to proliferate. A relationship between boron, IAA, and carbohydrate transport may occur, however, the relationship is apparently more complex than previously proposed. Middleton et al. (1980) reported that the transport of sucrose from cotyledons to the hypocotyl was increased by exogenous applications of auxins to the hypocotyl. If applications of B followed auxin treatments, the flux of carbohydrates to the hypocotyl was significantly (5x) increased (Middleton et al. 1980). These results suggest that B may sensitize tissues to auxins.

The Influence of Mycorrhizal Inoculation on IAA Levels, IAA Oxidase Activity and Peroxidase Activity

Mycorrhizal inoculation significantly increased the levels of IAA, and activity of IAA oxidase in plant roots; however, the levels of IAA here are considerably less than those reported by Sherwood and Klarman (1980) for mycorrhizal and nonmycorrhizal pine roots. It has been suggested that that an IAA content in mycorrhizae of 50 to 100 times that of nonmycorrhizal short roots has to be maintained in order to develop morphogenic characteristics of mycorrhizal short roots (i.e. dichotomous branching, lack of elongation, etc.). Mycorrhizal inoculated roots in boron fertilized control treatments were only three times greater in IAA concentrations than were the noninoculated seedlings of the same treatment, and the differences in IAA concentrations between inoculated and noninoculated B fertilized seedlings was considerably less. This may suggest that extremely elevated levels of IAA are not important in the maintenance of the mycorrhizal symbiosis, however, IAA levels 2-3 times greater for mycorrhizal plant roots are likely to be important in promoting the transport of carbohydrates to the root system and thus to the fungi.

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NITROGEN FERTILISATION AND ECTOMYCORRHIZAL FORMATION OF PINUS CARIBAEA (MORELET) SEEDLINGS

By

M. A. Amakiri and L. I. Ojobo

Keywords - Growth of pines, Ammonium sulphate, Ammonium nitrate, Phosphorus, Soil inoculum.

Introduction

The main obstacle to a large scale afforestation of Pines in Nigeria other than seed supply has been identified as the difficulty with mycorrhizal formation (Madu, 1967). But in addition, the optimum nutrient requirement, especially nitrogen, for the initial mycorrhizal infection has not been determined. This study investigates the effect of two sources of nitrogen fertilisation on the growth and ectomycorrhizal formation of Pinus caribaea (Morelet) seedlings.

Methods and Materials

Pinus caribaea seedlings, germinated in sterile top soil and washed sand, were pricked into polypots (13 cm x 8cm) containing top soil, bone meal, horn and hoof flakes and 3g single super-phosphate per 1kg soil. The mixture gave 206 ppmN. During pricking out, seedlings received soil inoculum collected from established pine plantations. After 8 weeks growth, N, at 22.43 kgN/ha and 44.87kgN/ha were applied as $(NH_4)_2SO_4$ and NH_4NO_3 to give total N in potting mixture as 216 ppm and 226 ppm respectively. The experimental design was randomised complete block, set up in the glass house, where five replicates served as blocks. At monthly intervals, the height, collar diameter, number of needles, number of mycorrhized roots (bifurcate short roots later forming corraloids) and dry weights of total plants dried to constant weight at 70°C were measured and percentage N and P in seedlings analysed.

Results and Discussions

N application increased growth of pine seedlings (Table 1). $(NH_4)_2SO_4$ applied at 44.87 kgN/ha significantly increased height and number of leaves. Treated seedlings significantly ($p = 0.05$) had higher dry matter yield than control. Mycorrhizal formation was higher with low N and least with high N (Table 2) 216 ppm N which gave best mycorrhizal frequency is higher than the 62 ppmN reported for lodge-pole pine (Ekwebelam and Reid, 1983). High N produced better growth, with lodging tendencies by seedlings, better root development but lower mycorrhiza (Fig. 1). Low N had less extensive root system. There was low N and high P in seedlings receiving high N and a high N in seedlings treated with low N. Regardless of other factors affecting ectomycorrhization in Pinus caribaea, results from this study show that the optimum level of N for healthy growth, good root development and high mycorrhization is between 216 ppm and 226 ppm.

Table 1. Effect of nitrogen fertilisation on the growth of Pinus caribaea seedlings 1/

N-Source	Level kgN/ha	Ht (cm)	Collar diameter (mm)	Dry wt (g)	No of leaves
$(NH_4)_2SO_4$	22.43	34.3b	1.3a	2.5a	24.1b
	44.87	35.7a	1.3a	2.3a	25.3a
NH_4NO_3	22.43	32.7b	1.2a	2.3a	24.8b
	44.87	33.9b	1.3a	2.3a	24.4b
Control	-	31.5b	1.1a	1.0b	23.1b

1/ Those means within a column not sharing common letter differ significantly (p=0.05) by LSD.

Table 2. Mycorrhizal frequency, %N and %P in Pinus caribaea seedlings receiving N-fertilization.

N-Source	Level (kgN/ha)	N in 0.5g seedlings (%)	P in 0.5g seedlings (%)	Mycorrhizal frequency (%)
$(NH_4)_2SO_4$	22.43	1.28	0.12	56.4
	44.87	0.93	0.17	51.6
NH_4NO_3	22.43	0.93	0.13	57.6
	44.87	0.70	0.21	51.0
Control	-	1.36	0.14	54.4

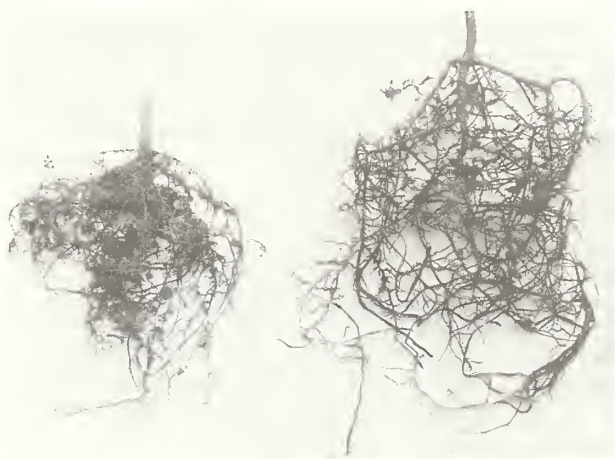

Figure 1. Root system of Pinus caribaea (with corraloid mycorrhiza) applied with low N (left) and High N (right) X1.

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INTERACTION OF NITROGEN, PHOSPHORUS AND MYCORRHIZAE INOCULATION ON NUTRIENT CONTENT AND GROWTH OF PINUS CONTORTA

By

J. Rousseau and C.P.P. Reid

Keywords--Pinus contorta, Suillus granulatus, nitrogen, phosphorus

Introduction

The commonly found increase in growth associated with ectomycorrhizae inoculation is often attributed to improved nutrient uptake. Although there is considerable evidence to suggest that ectomycorrhizae can enhance phosphorus uptake (Harley 1969), there is also widespread belief that ectomycorrhizae concomittently should enhanceuptake of nitrogen and other nutrients (Harley and Smith 1983, France and Reid 1983). The data of Hatch (1937) and more recently Reid et al. (1983) indicate that mycorrhizae development in conifers can increase foliar nitrogen concentration, further suggesting that mycorrhizae may enhance nitrogen as well as phosphorus uptake.

If the growth response associated with ectomycorrhizae development is in fact simply a result of enhanced mineral nutrition, then it should be possible to simulate the response to mycorrhizae by applying the appropriate combination of mineralnutrients to the growing media of nonmycorrhizal plants. The purpose of this study was to determine if the growth response of lodgepole pine seedlings to fungal inoculation under N and P deficient potting media could be similarly achieved by the addition of N and P, separately and in combination, to the media of non-inoculated seedlings.

Methods and Materials

Experiment 1
Seventy-five lodgepole pine (Pinus contorta) seedlings were grown in a high light intensity growth chamber for 7 weeks. The seedlings were thoroughly watered 3 times weekly with Hocking's solution modified to 10ppm and 1 ppm P. At the end of 7 weeks, 45 seedlings were subjected to 3 levels of nitrogen (10,50,100ppm) and 3 levels of phosphorus (1,10,20ppm) in a 3X3 factorial design with 5 replications. Additionally, 15 seedlings grown at the low N and P level (1oppm N, 1ppm P) and medium N and low P level (50ppm N, 1ppm P) were inoculated with the mycorrhizal fungus Suillus granulatus. At the end of 7 additional weeks the following parameters were measured on all seedlings: shoot and root weight, shoot/root ratio and foliar N and P concentrations.

Experiment 2
Experiment 2 was similar in design to experiment 1 with the exception that the treatments consisted of 4 levels of P(1,4,8,12ppm) and 2 levels of N(10,50ppm). In addition, all N and P treatments were further factored into inoculated and non-inoculated treatments (n=10).

Results and Discussion

The results from experiment 1 for N and P concentrations are presented in Figure 1 and results from inoculated seedlings are presented in Figure 2. It was found that the mycorrhizae response more closely resembled a phosphorus response than either a nitrogen response or a nitrogen-phosphorus interaction. Inoculated plants had greater foliar P concentration but decreased foliar N concentration than non-inoculated seedlings. A similar trend with fertilized non-inoculated seedlings was only observed with P application. Fertilization with N tended to have the reverse effect, i.e., N concentration was increased and P concentration was decreased. Fertilization with N and P tended to increase both foliar N and P concentrations. Results from experiment 2 are presented in Figure 3.

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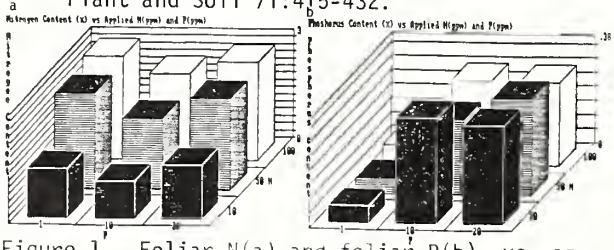

Figure 1. Foliar N(a) and foliar P(b) vs. applied N and P.

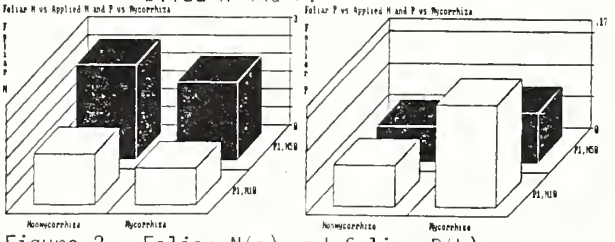

Figure 2. Foliar N(a) and foliar P(b) vs. applied N and P vs. mycorrhiza

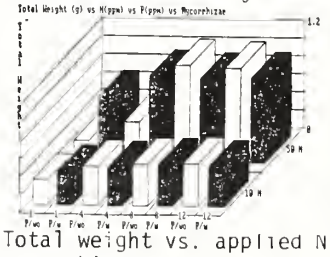

Figure 3. Total weight vs. applied N and P vs. mycorrhiza.

Scanning, Transmission, and Freeze Fracture Electron Microscopy of *Suillus granulatus* - *Pinus contorta* Ectomycorrhiza

By

D.E. Crowley and C.P.P. Reid

Keywords--T.E.M., S.E.M., Freeze fracture, membranes

ntroduction

Electron microscopy has provided much information on the morphology and ultrastructure of mycorrhizae. Different E.M. techniques provide different types of information. Scanning electron microscopy (S.E.M.) is especially useful for examination of surface features of mycorrhizae. Transmission electron microscopy (T.E.M.) allows examination of thin sections through mycorrhizal root tissue. Specific applications of these techniques have recently been reviewed (Brown and King 1982). However, another more recent technique which is especially useful for examination of membrane surfaces in both plants and animals, freeze fracture, has not yet been employed for examination of mycorrhizae.

In freeze fracture microscopy, a platinum replica of membrane surfaces is produced and examined using an electron microscope. In order to expose the membrane surfaces and produce the replica, the tissue to be examined is rapidly frozen by immersion in liquid freon at a temperature of -80°C. The tissue is then transferred to liquid nitrogen and placed in a device where it is fractured with a knife at -105°C under a high vaccuum. As the knife shatters the frozen tissue, the fracture plane preferentially follows membrane surfaces where resistance to breakage is less than through the frozen water in the cell cytoplasm. Fracture can occur not only along membrane surfaces, but also through the lipid bilayer, cleaving the membrane into two halves. The replica is produced by shadowing the surface with an ultrathin layer of platinum, followed by a thin layer of carbon over the platinum. Once the replica is produced, the tissue is thawed and digested away from the surface of the replica, which can then be mounted on a grid and viewed with an electron microscope.

Materials and Methods

S.E.M. and T.E.M. Excised mycorrhizal roots were fixed in 2% glutaraldehde in .1M cacodylate and dehydrated in an alcohol and acetone series. Roots for S.E.M. were critical-point dried in liquid CO_2 and these dried specimens coated in gold-palladium alloy to a thickness of 100 Å. Roots for T.E.M. were embedded in Epon-Araldite-DDSA plastic, sectioned, stained in lead citrate and viewed on a Phillips 200 microscope.

Freeze Fracture. Excised roots were fixed as above and infiltrated with glycerol (cryoprotectant). The roots were frozen in liquid freon, fractured in a Balzers 301. Tissue was digested from the replica using 1% sodium hypochlorite and the replica viewed on a Phillips 200 microscope.

Figure 1. Electron micrograph of replica of freeze-fractured roots of *Pinus contorta* ectomycorrhizal with *Suillus granulatus*. Replica is of Hartig net area with hyphae in cross-section. Note membrane surfaces of septa (s). 3000X

Results and Discussion

Photomicrographs of ectomycorrhizae using T.E.M. and S.E.M. techniques revealed typical ectomycorrhizal morphology. In photomicrographs of freeze-fractured roots, membrane surfaces of both plant and fungal cells were observed to contain numerous particles and pits at high magnification, which are replicas of membrane bound proteins in the lipid bilayer (Rash 1981). At lower magnification large areas of the Hartig net were observed (Fig 1). Three dimensional topography of the fractured surface was remarkably apparent in examinations of stereo micrographs.

All three E.M. techniques have specific applications for examining various aspects of mycorrhizal morphology. These results in which freeze-fracture techniques were employed for the first time in examination of ectomycorrhizae demonstrate the potential applications of freeze fracture for examining membrane surfaces of fungal and plant tissues in mycorrhizae. As further advances are made in specific applications of freeze fracture, such as the labeling of specific proteins using antibodies and postshadow labeling techniques (Rash 1981), freeze fracture will become even more useful for relating morphology to physiological function.

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Cytochemical Localization of p-nitrophenyl Phosphatase Activity in *Suillus granulatus*

By

D.E. Crowley and C.P.P. Reid

Keywords--ATPase, nutrient transport

Introduction

Two distinct classes of ATPase are currently recognized; the proton-translocating ATPase of bacterial, chloroplast, and mitochondrial membranes, and the transport-ATPases of eukaryotic cell membranes which pump ions other than protons (Firth 1978). Fungal plasmalemma ATPase, an electrogenic proton pump, appears to have properties in common with both of the above classes of membrane-bound ATPase (Slayman 1983). In fungi, protons are pumped out of the cell to generate a pH gradient and membrane potential, used to drive transport of sugars, amino acids, and other ions (Huschka et.1983). However, unlike other proton ATPases, fungal proton ATPase utilizes two steps in its reaction mechanism, forming a phosphorylated intermediate, similar to the Na^+-K^+-dependent ATPase of animal cells.

Cytochemical methods for specific localization of the Na^+-K^+-ATPase in animal cells have been developed and undergone extensive modification to assure specificity for Na^+-K^+-ATPase. It has been found that the second (dephosphorylation) step can utilize p-nitrophenyl phosphate as substrate and that this activity is K^+ dependent and ouabain sensitive (Bader and Sen 1966). Although the fungal proton-ATPase is similar in its reaction mechanism, the ability of fungal plasmalemma ATPase to use p-nitro phenyl phosphate as substrate has not been examined. This paper reports on localization of such activity in an ectomycorrhizal fungus using the cytochemical techniques of Mayahara et.al. (1980).

Methods and Materials

Ectomycorrhizal roots of *Pinus contorta* - *Suillus granulatus* were excised, and prepared for incubation in solution containing pNPP, levamisole (alkaline phosphatase inhibitor), DMSO, NaOH or KOH, glycine, and lead citrate according to the procedures of Mayahara et.al. (1980). Roots were incubated in solutions with and without K, and in the presence and absence of ouabain. After incubation, the roots were post-fixed in OsO_4, and prepared for electron microscopy.

Results

The phosphorus product of p-nitrophenyl phosphatase activity precipitates with lead from lead citrate to form lead phosphate deposits at sites of enzyme activity. These deposits are electron dense and allow localization of PNPPase activity within the cell. In this study, PNPPase activity was observed in all treatments on the cytoplasmic side of the plasmalemma of *Suillus granulatus* (Fig.1). No PNPPase activity was observed in the host plant root cells. Fungal PNPPase activity did not appear to be affected by the presence or absence of ouabain, or the presence or absence of K in the incubation medium.

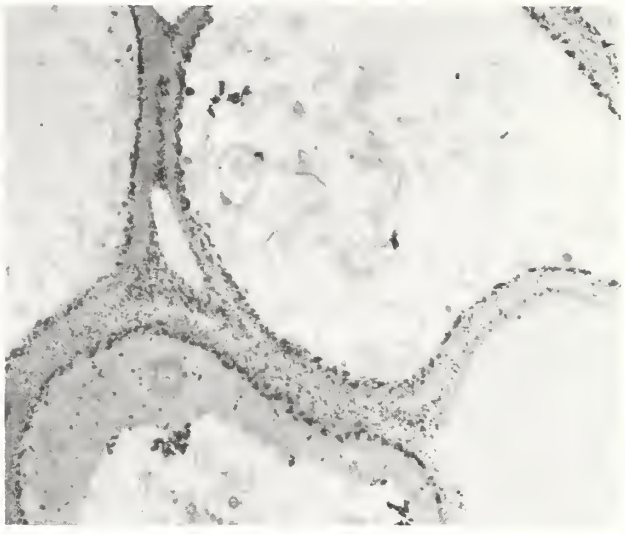

Figure 1. Localization of p-nitrophenyl phosphatase activity in *Suillus granulatus* ectomycorrhizal with *Pinus contorta*. Sites of enzyme activity can be seen as dark staining areas on the inner side of the plasmalemma where electron dense deposits of $PbPO_4$ precipitated when the fungus was supplied with PNPP in the presence of Pb-citrate.

Discussion

Localization of p-nitrophenyl phosphatase activity on the plasmalemma of *Suillus granulatus* provides cytochemical evidence for similarity between the fungal-proton-ATPase and the Na^+-K^+-ATPase of animal cells and the dissimilarity of fungal-proton-ATPase from other proton-ATPases. The lack of K-dependence of this activity is consistent with the data of Bowman and Slayman (1979) that previously demonstrated fungal ATPases are not stimulated by K^+ or Na^+.

A model has been presented for the function of fungal-proton-ATPase in the H^+-dependent cotransport of nutrients taken up by fungi (Slayman 1983). However, the molecular details of H^+-transport and H^+-cotransport systems are still unknown. Further work in this area will aid in understanding these mechanisms of nutrient transport in fungi.

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PREDICTION OF FINE ROOT PRODUCTION AND TURNOVER FROM ACCUMULATION AND DEPLETION OF STARCH

by

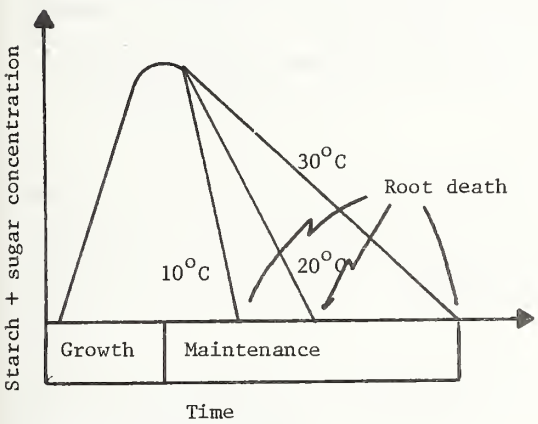
J.D. Marshall and R.H. Waring

Keywords--Pseudotsuga menziesii, fine roots, root turnover, starch, respiration, soil temperature

Introduction

Maintenance of a functioning fine root system requires large amounts of photosynthate. This can be attributed largely to the short lifetimes of the fine roots, which are replaced (turned over) two to three times per year. We hypothesized that lifetimes of fine roots are limited by their ability to meet maintenance requirements from the starch reserves in their tissues. Root lifetimes would then be determined by soil temperature because maintenance respiration rates increase exponentially with temperature.

Figure 1. Hypothesized starch and sugar dynamics of a fine root, showing differences in lifespan associated with root temperature.



Methods and Materials

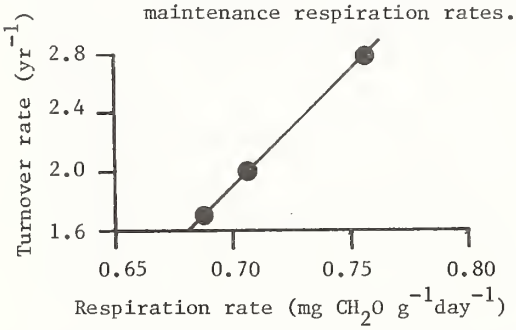
Two-year-old Douglas-fir (Pseudotsuga menziesii Mirb. (Franco)) seedlings with low mycorrhizal infection rates were transplanted into washed river sand in 550 cm^2 plastic tubes. Prior to budbreak, seedlings were transferred into controlled-temperature facilities in a growth chamber. Root systems were maintained at 10°C, 20°C, and 30°C, air temperatures at 21°C. Shoots of half the seedlings were covered to exclude light and halt photosynthesis, stopping the translocation of photosynthate into the root system. Seedlings were harvested at intervals and sugar and starch were extracted with 80% ethanol and 35% perchloric acid, respectively. Hexose concentrations were determined by the anthrone reaction.

Results and Discussion

At 20°C and 30°C, no starch was deposited in the root systems, whether seedlings were growing in the light or maintained in darkness. At 10°C, however, starch was deposited in roots of seedlings growing in the light. The increase in starch concentration was proportional to fine root growth, which suggests that the starch was being deposited in the growing roots.

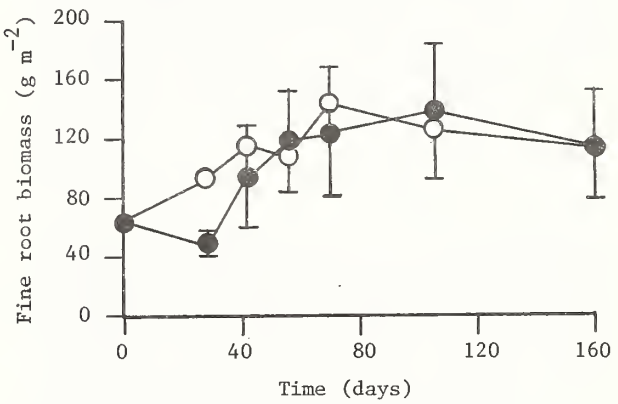
The relation between soil temperature and turnover of fine roots can be examined using data collected in field studies. Roots sampled in these studies were predominantly mycorrhizal. Maintenance respiration rates estimated from soil temperature were closely related to fine root turnover measurements of Santantonio (1982).

Figure 3. Regression of fine root turnover rates (Santantonio 1982) against estimated maintenance respiration rates.



Root biomass can also be predicted from root starch concentration and soil temperature data by assuming that all starch deposition occurs in new roots.

Figure 4. Predicted (○) and measured (● ±SE) fine root biomass from data of Ericsson and Persson (1980).



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EFFECTS OF MYCORRHIZAS AND pH ON NITROGEN UPTAKE BY NW CONIFEROUS SEEDLINGS

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Keywords-Pseudotsuga menziesii, Picea sitchensis, Tsuga heterophylla, Hebeloma crustuliniforme, pH, ammonium, nitrate

Introduction

Nitrogen availability is the most important nutrient factor limiting forest growth in the Pacific Northwest of North America, where coniferous species respond positively to nitrogen fertilization. Acidic NW forest soils commonly have reduced nitrification and mineralization rates, which makes ammonium (NH_4^+) the prevalent form of inorganic nitrogen, although nitrate (NO_3^-) is also present in low concentrations. Mycorrhizae are presumed to enhance nitrogen acquisition by coniferous roots and this research reports on effects of the ectomycorrhizal fungus Hebeloma crustuliniforme on ammonium and nitrate uptake by 3 important NW coniferous species - Douglas-fir, Sitka spruce and western hemlock.

Study of ammonium or nitrate acquisition is complicated by several factors. Protons released during ammonium uptake or hydroxyl ions released during nitrate uptake substantially affect ambient solution or rhizosphere pH. These pH changes can alter subsequent uptake of ammonium and nitrate as well as other ions and affect availability of nutrients. Biochemical and biophysical processes internal to the cell which control ambient and cellular pH have been shown to vary with external pH. Therefore external pH should be maintained at constant values while studying nitrogen uptake, as was done is this research.

Methods

Seedlings of Douglas-fir, Sitka spruce and western hemlock were grown in peat/vermiculite in plastic tubes in a greenhouse. After seed germination and 2-3 months growth, half of the seedlings were inoculated with the ectomycorrhizal fungus Hebeloma crustuliniforme. Nine-month old seedlings were removed from the tubes, and roots were gently washed free of the medium. Roots of intact seedlings were pretreated in dilute nutrient solution with 0.04 mM ammonium or nitrate for 48 h, and aeration and supplemental lighting. Roots were then placed in uptake solutions containing N-15 labeled ammonium 1.4 mM) or nitrate (0.7 mM) for 3-4 h in a series of pH solutions from pH 2.5-7.5. After a 15 min desorption period, roots were dried, weighed, digested and analyzed for total N (microKjeldahl) and N-15 (mass spectrometry). Uptake solutions were sampled at the beginning and end of the uptake periods. Uptake or release of Ca^{2+} or K^+ was calculated as the difference between initial and final nutrient concentrations as determined by atomic absorption. Release of H^+ (ammonium experiments) or OH^- (nitrate experiments) was assumed to equal the amount of titrant dispensed during the uptake period.

Results and Discussion

Nitrate Experiments: While both coniferous species and mycorrhizae did affect nitrate uptake rates, the major effect on nitrate uptake rates was solution pH. Nitrate uptake rates generally increased with increasing pH from pH 2.5 to 7.5, as did calcium flux rates which changed from efflux at low pH to uptake at higher pH levels. Potassium was released from the roots at all pH levels. Hydroxide ion release rates decreased with increasing pH, resulting in hydrogen ion release above pH 5.5. Mycorrhizal roots often released fewer hydroxide ions per nitrate ion taken up than nonmycorrhizal roots, leading to the suggestion that mycorrhizae may act as rhizosphere buffers. Among the three coniferous species, Douglas-fir roots released more hydroxide ions per nitrate taken up than did western hemlock; Sitka spruce values were intermediate. These apparent species effects may be related to the less acidic mineral soil environment where Douglas-fir roots are often found and to the more acidic forest floor environment where western hemlock roots grow.

Ammonium Experiments: Ammonium uptake rates decreased with decreasing pH and were accompanied by decreasing H^+ release to the external solution. Mycorrhizae enhanced ammonium uptake and generally released fewer hydrogen ions per ammonium ion taken up than did nonmycorrhizal roots. This altered stoichiometry in mycorrhizal seedlings could mediate pH changes in the rhizophere of soil grown plants. During ammonium uptake, calcium ions were released at low pH and taken up at higher pH. Potassium efflux occurred at all hydrogen levels, with less efflux at higher pH. The mycorrhizal association generally increased calcium uptake or decreased calcium and potassium efflux rates compared with nonmycorrhizal seedlings.

In summary, mycorrhizae increased uptake of both ammonium and nitrate, but had a greater influence on ammonium uptake rates. Mycorrhizae generally lowered the OH^-/NO_3^- ratio (nitrate) or the H^+/NH_4^+ ratio (ammonium) for coniferous species, suggesting that mycorrhizae may act as rhizosphere buffers. Nitrogen can be taken up by mycorrhizal roots with less change in rhizosphere pH, an effect also noted in soil studies (Bledsoe and Zasoski, Plant & Soil 71, 445-454, 1983). This research supports the concept that mycorrhizae benefit plants by increasing ion uptake, but this effect may be more important for less mobile ions such as ammonium and less important for more mobile ions such as nitrate. This research will be published in the Canadian Journal of Forest Research.

RUBIDIUM TRANSFER RATES AND STORAGE IN MYCORRHIZAL CONIFEROUS ROOTS

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Keywords-Pseudotsuga menziesii, Picea sitchensis, Tsuga heterophylla, Hebeloma crustuliniforme, potassium, compartmental analysis, uptake

Introduction

The technique of compartmental analysis of radioisotope elution from plant tissues has been used extensively to determine the number of compartments participating in solute exchange, to estimate the solute content of each compartment and to calculate fluxes of the solute between compartments. Compartmental analysis of solute elution from mycorrhizal roots can obviate the need for physical separation of fungal mantle from host roots of ectomycorrhizal plants. In addition to estimating gross storage and transfer rates of higher plant and mycobiont with sheath and Hartig net included, estimates can be made of nutrient fluxes and pool sizes between and within each compartment of the mycorrhizal association. This research reports on the uptake and efflux of 86-Rb, used as a tracer for potassium, by intact roots of mycorrhizal and nonmycorrhizal seedlings of three conifer species. Compartmental analysis was used to develop a model describing nutrient storage and transfer rates.

Methods

Seedlings of Douglas-fir, Sitka spruce, and western hemlock were grown in peat/vermiculite in plastic tubes in a greenhouse. Four month old seedlings were inoculated with the ectomycorrhizal fungus Hebeloma crustuliniforme. After 7-8 months, seedlings were removed from the tubes and the roots gently washed. Roots of intact seedlings were pretreated for 24 h in dilute, aerated nutrient solution, then transferred to aerated radioactive loading solutions containing 0.07 uM 86-RbCl (sp. act. = 0.05 uCi/uMol K), 0.1 mM KCl and 0.5 mM $CaSO_4$, pH 5.0. During the 18 h loading period, seedlings were maintained at 18° C under continuous light. After loading, roots were removed, washed for 5 s in dilute nutrient solution, then transfered to 30 ml syringes fitted with valves for drainage of solutions. Successive 18 ml volumes of unlabeled efflux solution were added to the syringe and incubated with the roots (or in some cases with fungal material alone) for sufficient time to allow 86-Rb elution. Initially incubation periods were 2 min, gradually lengthened to 1 h (total efflux period = 10 h). After 10 h, roots were weighed, dry-ashed and total radioactivity determined. An additional short-term uptake experiment was done to measure 86-Rb fluxes from the external solution into the cytoplasm. Uptake was measured for 30, 60 and 90 min. Uptake was corrected for cell wall + free space adsorption by parallel experiments at 2° C. A nonlinear regression method was developed for determining the initial isotopic contents and isotopic exchange rate constants for three cellular compartments. This method uses a regression equation with the form of a general exponential decay function.

Results and Discussion

The Rb^+ uptake rate for the fungus alone was 11 times greater than the average rate for all nonmycorrhizal seedlings, suggesting that a small amount of fungal biomass in mycorrhizal roots can have a large effect on nutrient uptake rates. Rubidium uptake rates were significantly greater for mycorrhizal than for nonmycorrhizal seedlings. These increases were 1.9, 3.0 and 3.2 times greater for mycorrhizal Douglas-fir, Sitka spruce and western hemlock respectively. Greater uptake rates for hemlock relative to Douglas-fir may have been due in part to the higher percentage of mycorrhizal root tips which were observed for hemlock. Less than 5% of the absorbed Rb was translocated to the shoots during the 18 h loading period. Although there was a trend toward increased Rb translocation by mycorrhizal seedlings, this was not statistically significant.

Using the 3-exponential equation, half-times and apparent initial isotopic contents of 3 cellular compartments were estimated. These 3 compartments were assumed to be the CW+FS (cell wall + free space), cytoplasm and vacuole for nonmycorrhizal roots. For mycorrhizal roots, these 3 compartments were assumed to be the average of both root and fungal CW+FS, cytoplasm and vacuolar compartments. Half-times were about 2 min for the CW+FS, 20-45 min for the cytoplasm and 400-6,000 min for the vacuole. The mean half-times were increased substantially by the presence of the fungus, particularly for the vacuolar compartment (380% increase). This effect was probably due to the presence of additional fungal vacuolar compartments. As for the half-time data, mycorrhizae also increased the isotopic contents for all compartments, particularly the vacuole (190% increase).

Using compartmental analysis, fluxes and pool sizes were estimated. Generally there was a net potassium uptake by mycorrhizal roots and net potassium release by nonmycorrhizal roots. We recognize that K+ release must be a short-term phenomenon since these plants accumulate K+. Potassium fluxes are quite variable and can change from uptake to efflux depending on external and internal conditions. Flux data showed that mycorrhizae had lower fluxes from the vacuole to the cytoplasm so that storage in the vacuole was enhanced, while in nonmycorrhizal roots K+ was transfered from the vacuole to the external solution. A model of K+ fluxes in these roots was developed and illustrates rapid rates of both K+ uptake and release. Of 37 units taken up, only 3% remain in the roots, stored in the fungal vacuoles. This research is being published in Plant Physiology as a series of two papers.

ORGANIC NITROGEN UPTAKE BY AXENICALLY-GROWN MYCORRHIZAL CONIFEROUS ROOTS

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Keywords-Pseudotsuga menziesii, Hebeloma crustuliniforme, organic nitrogen, uptake

Introduction

The importance of nitrogen to forest productivity has been well established. This has stimulated interest in understanding mechansims by which nitrogen is conserved in forest ecosystems. Internal nitrogen recycling and nitrogen immobilization in the forest floor are two possible mechanisms. Mycorrhizal roots are concentrated in the forest floor, but the forms of nitrogen taken up by tree roots are not certain. Many studies of nitrogen cycling have not evaluated the importance of organic nitrogen, assuming that all nitrogen uptake by roots is in inorganic form. However the pool of soluble organic nitrogen and fluxes through this pool may be substantial. We hypothesize that mycorrhizal roots can compete with other soil microorganisms and may take up a significant amount of organic nitrogen. This study investigates the first step - can mycorrhizal roots take up organic nitrogen, specifically as amino acids?

Methods

Douglas-fir (Pseudotsuga menziesii) seedlings were grown from seed in large glass tubes axenically in peat/vermiculite with nutrients and MMN added. Half of the seedlings were inoculated with the fungus Hebeloma crustuliniforme. After 6-9 months, seedlings were removed and roots carefully washed. Seedlings were pretreated for 40 h in 0.5 mM $CaSO_4$, pH 5.5. Amino acid uptake was measured by placing roots of intact seedlings in aerated uptake solutions containing 0.5 mM $CaSO_4$, 5.0 mM glycine, and C-14 glycine. After varying uptake periods (10 min - 24 h), roots were desorbed for 15 min at 2^{o} C, weighed, frozen and ground in liquid N_2, and extracted with 80% ethanol. Extracts were chromatographed on thin-layer chromatograms; autoradiograms were made.

Results and Discussion

When Douglas-fir seedlings were grown axenically, mycorrhizal seedlings produced more roots than shoots, while nonmycorrhizal seedlings produced more shoots than roots. Mycorrhizal roots averaged 50-80 root tips per seedling and showed extensive mantle development. During uptake periods, glycine was taken up and H^+ ions were released to the external solution. Solution pH was maintained continuously at 5.5 with a pH stat unit. Maximal glycine uptake rate for nonmycorrhizal roots was 0.55 pMol/mg fresh wt roots/hour and occurred after 4 hours exposure to glycine. The maximal rate for mycorrhizal roots was 0.83 pMol/mg fresh wt roots/hour and occurred after 12 hours exposure to glycine. Mycorrhizal roots took up greater quantities of glycine, although both mycorrhizal and nonmycorrhizal systems appeared to be saturated after 12 hours.

During all uptake periods, a substantial amount of glycine was present in the cell wall+free space and in surface water on the roots; multiple desorption periods at 2^{o} C in unlabeled glycine were required to exchange C-14 labeled glycine. During short uptake periods, this exchangeable glycine was 9-10 times greater than the internally absorbed glycine.

These preliminary experiments suggest the following conclusions:

1. Douglas-fir seedling roots take up the amino acid glycine and this uptake is enhanced by the presence of the mycorrhizal fungus Hebeloma crustuliniforme.
2. Glycine is taken up by seedling roots for about 12 hours, after which time little more glycine is absorbed by the roots, whether mycorrhizal or nonmycorrhizal.
3. A desorption period of at least 15 minutes with at least 3 separate rinses is recommended, particularly for mycorrhizal roots, where the cell wall + free space exchange volume is greater than in nonmycorrhizal roots.
4. During uptake periods as short as 60 min, glycine is rapidly converted into 3-7 other amino compounds; possible differences between mycorrhizal and nonmycorrhizal roots are being examined.

REGULATION OF ACID PHOSPHATASE ACTIVITIES OF SELECTED SPECIES OF ECTOMYCORRHIZAL FUNGI

By

R.K. Antibus, C.J. Kroehler, and A.E. Linkins

Keywords--Cenococcum, Km, orthophosphate, pH, phytate, temperature, Vmax

Introduction

The increase in phosphorus accumulation and improvement of phosphorus status of higher plants caused by symbiotic association with mycorrhizal fungi has been well documented. The diffusion rate of inorganic phosphorus to the root surface is the factor limiting phosphorus uptake in most soils, and a widely accepted explanation for the ability of mycorrhizae to enhance phosphorus uptake is that extramatrical hyphae extend the absorbing surface of the root (3).

The demonstration that ectomycorrhizal fungi have nonspecific surface acid phosphatases capable of hydrolyzing a number of organic phosphate compounds (2) invited speculation that mycorrhizae may contribute to the phosphorus nutrition of host plants by producing inorganic phosphorus from organic sources otherwise unavailable to the higher plant root. The objectives of this study were to characterize surface acid phosphomonoesterases of several ectomycorrhiza forming isolates and to investigate various factors that might play a role in the regulation of phosphatase production and activity.

Specifically, experiments were designed to determine the effects of temperature acclimation on the activation energy of the surface phosphatases; to investigate the regulation of surface phosphatase activity by external pH; to determine the kinetic constants (Km and Vmax) for the surface and excreted phosphatases of these isolates; and to determine the relationship between kinetic constants and concentrations of inorganic or organic phosphorus in the culture medium.

Methods and Materials

The fungi used in this study were isolates of Entoloma, Hebeloma pusillum, and Cenococcum geophilum from Alaska and Maryland (1). Plugs taken from plates of the isolates were allowed to grow for 35 days in 50 ml liquid medium at 20 C. Individual mycelial mats were assayed for surface acid phosphatase activity using p-nitrophenyl phosphate (pNPP) as substrate (4). Plugs were incubated at 20 C in 2 ml buffer-substrate mixture (pH 5.5) for three hours. An aliquot from each reaction tube was added to 0.5 N NaOH and the optical density of the samples was read at 410 nm on a spectrophotometer. Assayed plugs were lyophilized and weighed on an electrobalance.

For temperature acclimation studies, isolates were grown at 12 or 20 C and assayed for phosphatase activity at 1, 5, 10, 15, 20, 25, and 30 C with saturating concentrations of pNPP. For pH investigations, mycelial plugs were assayed at pH values from 2.5 to 6.0. To determine the effect of different concentrations of inorganic and organic phosphorus on phosphatases, phosphorus was provided in the culture medium as orthophosphate (KH_2PO_4) or phytic acid (inositol hexaphosphate) at final concentrations of 2, 50, 500, and 1000 micromolar. Mycelial mats were assayed at 5, 10, 25, 50, and 100 mM pNPP. Samples from the culture media were similarly assayed. Kinetic constants were determined from Eadie-Hofstee transformations of the data and tested for significant differences by analysis of variance and multiple range tests.

Results and Discussion

Activation energies for surface acid phosphatases varied from 12.2 to 20.0 kcal $mole^{-1}$ for the isolates examined. Activation energies were not significantly affected by growth of isolates at different temperatures (12 and 20 C). Phosphatase activity was highest at pH 5.0 for four of six isolates assayed. One isolate showed little response to changes in external pH.

Kinetic parameters (Km and Vmax) demonstrated marked differences among the isolates examined. Although significant differences were found among Km and Vmax values determined for isolates grown at different concentrations of organic and inorganic phosphorus, the values do not correlate with increasing or decreasing levels of inorganic or organic phosphorus. Kinetic constants determined for media samples also were unrelated to phosphorus in the media. Dry weight accumulation was little affected by the concentration or type of phosphorus in the medium; only when phosphorus was totally lacking was growth significantly reduced.

It has been suggested that phosphatases play a role in the solubilization of organic forms of soil phosphate. Our results, however, fail to establish a clear link between levels of organic phosphorus and phosphatase production or activity. Although the ecological significance of surface acid phosphatase activity is unclear at present, our findings show that different isolates of a species and different species demonstrate significant differences in acid phosphatases in terms of kinetic parameters and pH and temperature response.

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EFFECT OF MYCORRHIZAL FUNGI ON GROWTH AND DEVELOPMENT OF ROOTS IN SEEDLINGS OF PINUS RESINOSA.

By

Chin S. Yang and Hugh E. Wilcox

Keywords--Pisolithus tinctorius, Suillus subluteus, E-strain fungus, root growth and development

Introduction

Ectomycorrhizal fungi had been shown to alter root morphogenesis (Slankis, 1973). Much of this information is based on qualitative description, and few quantitative data are available on whether mycorrhizal fungi do cause alteration in root system in Pinus.

Using a culture tube technique (Yang and Wilcox, 1984), we examined root morphogenesis of red pine (Pinus resinosa) under influence of three mycorrhizal fungi. Quantitative results are presented to demonstrate the influence of each fungus on the root system of red pine seedlings.

Materials and Methods

Detailed of the experimented procedures have been described in Yang and Wilcox (1984). Three mycorrhizal fungi, Pisolithus tinctorius (PT), Suillus subluteus (SS), and BDG, an E-strain fungus (Wilcox et al., 1974) were used to inoculate 4-week-old red pine seedlings. Twelve seedlings were used for each treatment.

Results and Discussion

The slow-down in primary root elongation after 7 wk (Fig. 1) corresponding to the accelerated growth of long laterals (Fig. 2) indicated that an internal coordination between the primary root and long laterals may be at work. However, this pattern is not clear in the PT treatment. The inoculation with PT slows down the elongation of the primary root and suppress the growth of long laterals.

All treatments produced the first short root in 4 wk, and the numbers of short roots increased steadily afterward (Fig. 3). However, SS and BDG have larger increases and result in higher total short roots and short-to-long-root (S-L-R ratio) (Table 1), but did not reduce long root length. PT does not produce more short roots, but results in the highest S-L-R ratio due mainly to the smaller long root system caused by the fungus. These results show that mycorrhizal fungi do change root growth and development but may not follow the proposed pattern described in Slankis (1973). Individual fungus may show different effects on root morphogenesis.

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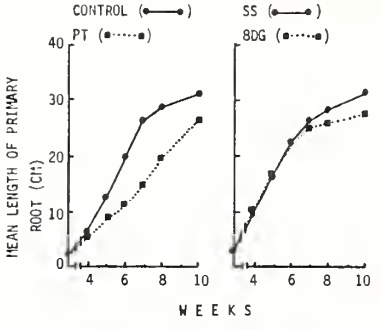

Figure 1. Growth curves showing mean lengths of the primary root in red pine seedlings with or without mycorrhizal fungi.

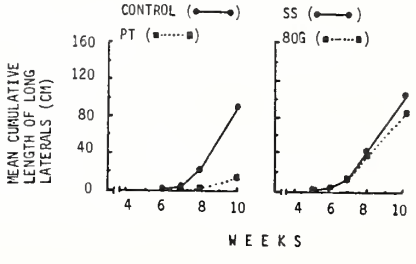

Figure 2. Mean cumulative lengths of long laterals in red pine with or without mycorrhizal fungi.

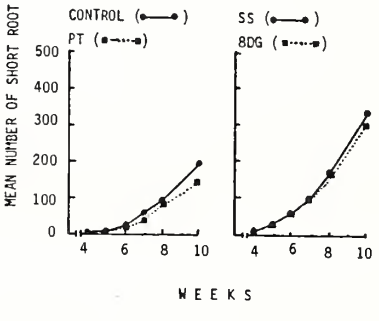

Figure 3. Mean numbers of short root in seedlings with or without mycorrhizal fungi.

Table 1. Total long root length, number of short roots, and short-to-long-root ratio of red pine seedlings inoculated with mycorrhizal fungi after a ten-week period.

	Mean total length of long root (cm)	Mean total short roots	short-to-long-root ratio (short roots per cm of long root)
Control(9)*	$122.28 \pm 13.70_{a}$	$195.33 \pm 23.04_{bc}$	$1.63 \pm 0.12_{c}$
SS (9)*	$137.91 \pm 18.97_{a}$	$333.89 \pm 45.94_{a}$	$2.46 \pm 0.09_{b}$
PT (8)*	$41.49 \pm 6.45_{b}$	$143.88 \pm 14.77_{c}$	$3.62 \pm 0.20_{a}$
8DG (11)*	$114.47 \pm 18.05_{a}$	$302.45 \pm 53.73_{ab}$	$2.56 \pm 0.23_{b}$

Values followed by the same letters are not significantly different at the 1% level according to Duncan's multiple range test.

* Numbers in parentheses are seedlings measured.

EFFECT OF SOME CULTURE CONDITIONS ON UTILIZATION OF CARBON SOURCES BY PISOLITHUS TINCTORIUS

By

W. A. Taber and R. A. Taber

Keywords--respiration, hydrolysis, radioactive sugars, induction

Introduction

Some recent data appear to support the earlier hypothesis that ectomycorrhizal fungi use only a few carbon sources (Hacskaylo 1973, Taber and Taber, 1982) while others suggest that many carbon sources are used (Lamb 1974, Smith, 1982). Assessments of growth are subjective and many factors can influence growth. Some examples are: length of incubation, strain differences, media composition, nutrient carry over from inoculum, history of inoculum, pH, and contamination of carbon sources. Some results of an ongoing study designed to further characterize carbon source utilization are herein reported.

Materials and Methods

Cultures were grown stationary at 28°C in a previously-described synthetic medium (Taber and Taber, 1982). All soluble carbon sources were recrystallized or precipitated. Respiration was measured in a Warburg respirometer using ^{14}C-labeled sugars; utilization, as nmoles, was calculated from radioactivity in respired CO_2 by references to combusted and counted aliquots of sugar solutions used in the studies.

Results and Discussion

Glucose and the dimer cellobiose were readily used for growth at pH 4.5, 5.4, and 6.3 while the dimers sucrose, maltose, and trehalose, and the hexose, fructose, were used (albeit poorly) only at lower pHs. The hydrolytic enzymes acting on these dimers no doubt have an optimal pH in the acid range; thus the capacity to use them would not be detectable when using a near-neutral pH medium. Use of a second carbon source as malt extract or acidogenic nitrogen source could allow pH to drop to a favorable range. Mycelia grown on glucose could respire sucrose and fructose but could not grow readily on them. Mycelia respired the glucose moiety of sucrose but not the fructose moiety, even though free fructose was respired. (Table 1).

Fructose probably is not used because the invertase transfers it to fructose, producing poorly-used fructose oligosaccharides, rather than to water residue producing free fructose. Data, not presented, suggest that high (i.e. conventional) concentrations of fructose repress certain of fructose enzymes.

Sucrose and fructose utilization are increased somewhat by 4 mMolar glucose but not by 0.4 mM. Abundant utilization of cellobiose, the dimer of cellulose, is not explainable; cellulose is not used by any of 3 strains examined.

Various pHs of experimental media were produced by adjustment with KOH and phosphate buffer (media were adjusted with KCl to have identical K^+ concentrations). Results of factorial design experiments indicated that inability to grow at pH 6.7 was not due either to K^+ concentration or to phosphate buffer concentration. Certain concentrations of phosphate can be inhibitory, however (Giltrap and Lewis, 1981; Hung and Trappe, 1983).

P. tinctorius possesses a high endogenous respiration; exogenous respiration is aided by washing and starving mycelia. During respiration, some of the glucose carbon is polymerized.

Table 1. Respiration of sugars based on radioactivity in respired CO_2

14 C-Carbon source	nmoles sugar per hour per mgm (dry wt) mycelium
Glucose	5.66 ± 0.16
Fructose	8.47 ± 2.90; 7.8 ± 1.3
Fructose + unlabeled glucose	6.94 ± 1.65
Sucrose (14C-glucose)	0.47 ± 0.08; 0.51 ± 0.05
Sucrose (14C-fructose)	0.09 ± 0.01; 0.08 ± 0.001

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ECTOMYCORRHIZAL-FUNGUS MEDIATED NUTRIENT TRANSFER BETWEEN TRADESCANTIA AND PINUS

By

R. A. Taber, and W. A. Taber

Keywords--spiderwort, pine, longleaf pine, carbon nutrition

Introduction

Previous observations on VAMF in spiderwort (Tradescantia) in Alabama (Taber and Strong 1982) prompted the examination of spiderworts growing in Texas. Spiderworts are common weeds and occur on a variety of soil types.

Methods and Materials

Spiderwort plants were uprooted and transported to the laboratory on ice where the roots were examined under a stereomicroscope (40x) and cleared (Phillips and Hayman 1970) for observation of VAMF. The observation that fine pine feeder roots were entwined around the spiderwort crown and root system suggested closer examination of the relationship between these two plants. The close association of these two root systems suggested some beneficial interaction between these plants. The possibility of nutrient transfer between plant species was considered and this hypothesis was tested by injecting 10-100 µCi ^{14}C sucrose into each of the stems of 8 spiderwort plants and also into exposed but intact pine roots leading up to 8 other spiderwort plants. The presence of radioactive carbon from the ^{14}C sucrose was detected by autoradiography after freeze drying the injected systems.

Results and Discussion

Spiderwort plants growing in East Texas were non-mycorrhizal. Pine roots were found not only encircling the spiderwort crown area, but also commonly grew into the mucilaginous spiderwort rhizosphere and down onto the rhizoplane. Long fine pine feeder roots grew in amongst spiderwort root hairs. The ectomycorrhizal symbiont on the pine produced abundant hyphae which ramified in the spiderwort rhizoplane. Fine pine roots were so tightly appressed to the spiderwort root surface that they had to be extricated individually under the microscope.

Injection of ^{14}C sucrose into the spiderwort stems resulted in the presence of radioactivity in the small fine pine feeder roots that had been pulled from the spiderwort rhizoplane. Radioactivity was also detected in spiderwort stems in situ when sucrose was injected into excavated pine roots. Control plants showed no radioactivity.

This information indicates the fact that nutrient transfer can occur between plants in natural forest ecosystems between trees and herbaceous plants and further, appropriate rapidly-growing herbaceous plants could conceivably be used as satellite nurse plants for ectomycorrhizal trees in production systems.

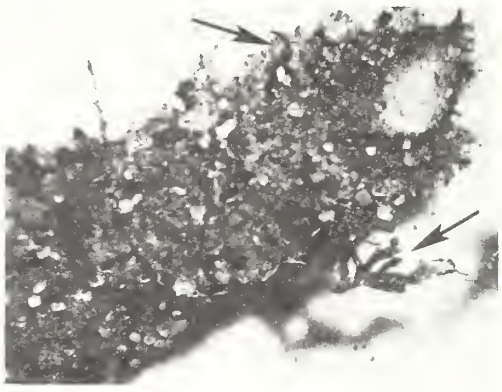

Figure 1. Closeup of spiderwort root showing adhering sand particles between root hairs in mucilaginous exudate. Note small pine roots at arrows.

Figure 2. A,B. Small pine roots pulled from spiderwort roots. Note proliferation of ectomycorrhizal fungus hyphae (A at arrow).

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EFFECT OF ACIDIFICATION OF CALCAREOUS SOIL ON THE PROLIFERATION OF ECTOMYCORRHIZAL *CARYA ILLINOENSIS* ROOTS

By

T. D. Riley, R. Taber, L. Fenn and J. Neck

Keywords--zinc, pecan, pH

Introduction

Zinc deficiencies commonly occur in pecans (*Carya illinoensis* (Wang) K. Koch) grown on calcareous soil of the Southwest. Soils in the middle Rio Grande Basin are alkaline, causing surface applied $ZnSO_4$ to be generally unavailable for plant uptake. The objectives of this study were to investigate the effectiveness of H_2SO_4 treatments to alter soil properties and the influence on ectomycorrhiza to promote nutrient uptake.

Methods and Materials

Two trenches were dug on opposite sides of all test trees into which acid and nutrients were applied. The trenches were 5 meters in length, 20 cm deep and 30 cm wide. Four treatments were applied: (1) zinc sulfate (4.5 kg) to the bottom of each trench, (2) zinc sulfate (4.5 kg) with 95 liters of concentrated sulfuric acid to each trench, (3) foliar spray of zinc sulfate, 1.4 kg in 379 liters of water, (4) trenches only as a control.

Soil pH was determined from a 1:1 water:soil mixture. Soil samples were taken 1, 2, 3, 4 year(s) after initial treatment to determine pH and nutrient levels. Roots were collected 4 years after initial soil treatments. Roots were prepared and observed under a light and scanning electron microscope for mycorrhizal infection.

Results and Discussion

Soil pH in the acidified zone dropped from 8.0 to approximately 4.3 at the end of the first year following initial treatment. No small roots were found in the treated area. Soil samples in the second year gave similar results. During the third year, root proliferation was occurring near the periphery of the acidified zone. By the fourth year, soil pH levels approached 6.5. Root proliferation at a soil depth of 15 cm from the $ZnSO_4$ plus H_2SO_4 treatment was greater than the control. Root growth from the other two treatments were not visibly different from the control. Results of soil chemistry, leaf tissue analysis, nut quality and yields are published elsewhere (Fenn et al., 1982; Malstrom et al, 1984).

Close observation of root samples, collected at the end of the fourth year, with a scanning electron microscope revealed a greater level of ectomycorrhizal development in the $ZnSO_4$ plus H_2SO_4 treatment than the control. Nutrient analysis of the rhizosphere soil and identification of the ectomycorrhizal species is under investigation.

Maintaining appropriate soil pH should maximize root efficiency and plant growth. Preliminary tests have shown that acidification of calcareous soil can increase zinc solubility in a limited area of the rhizosphere (Fenn et al., 1982). The area of the root zone requiring acidification to increase zinc availability is yet to be determined. However, these results support previous findings that pecans grow best at soil pH levels near 6.5 and indicate that ectomycorrhizae respond favorably to the acidification treatment (Sparks, 1977).

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ECTOMYCORRHIZAL FUNGI AND IBA EFFECTS ON FRUIT ROOTSTOCK ROOTING

By

B. Branzanti, G. Cristoferi, A. Zocca, and A. Zambonelli

Keywords: *Hebeloma crustuliniforme*, *Laccaria laccata*, auxins, abscissic acid, wheat coleoptile

Introduction

Hardwood cuttings of M 106 apple rootstock are difficult to root even if previously treated with rooting hormones. Some mycorrhizal fungi enhance the rooting ability of woody plant cuttings, Lindermann and Call (1977) who advance the hypothesis that some of these fungi may release growth hormones such as auxins, cytokinins, gibberellins as well as vitamins which stimulate root and shoot growth of tree seedlings before the formation, or in the absence of mycorrhizae (Slankis, 1973). The present trial is therefore designed to determine whether the addition of ectomycorrhizal inoculum to the rooting medium of the M 106 apple rootstock influences rooting ability of hardwood cuttings. The fungi inoculum effects were then compared to those of IBA-treated cuttings.

Methods and Materials

The fungi inocula were prepared according to the procedures described by Molina (1979). These inocula were then added to the rooting medium 50% vermiculite: 50% peat-moss in a 1:5 ratio inoculum:rooting substratum.

Hardwood cuttings of apple M 106 roostock (free from known virus disease) were collected in mid-February and stored at 4 C for 40 days. The cuttings were then divided into four groups. One group was placed in *Laccaria laccata*-inoculated soil, one in *Hebeloma crustoliniforme*-inoculated soil and another in uninoculated medium. The fourth group, previously treated with IBA powder at 2000 ppm, was also planted in untreated medium. All mediums were heated.

The auxin promoters and ABA inhibitors were extracted from apical and basal portions of the cuttings and assayed by coleptile straight growth test after the method described by Filiti and Cristoferi (1977).

Results and Discussion

The addition of *Laccaria laccata* to the rooting medium stimulated root initiation of the cuttings. 30 days after planting, 30% of the *Laccaria laccata*-inoculated cuttings achieved rooting. No rooting was observed in control and IBA-treated cuttings during the same period.

Figure 1. *Laccaria laccata*-treated cuttings (left). Untreated cuttings (right).

25 days later, rooting was almost four times higher in the *Laccaria laccata* cuttings than in control and three times that of IBA-treated cuttings.

Bioassays revealed that inocula of both fungi induced strong but slightly different modifications in hormonal levels. More drastic in the *Laccaria laccata* cuttings, these changes were less marked in the *Hebeloma* group, which may explain why *Hebeloma* had no influence on rooting. 30 days after inoculation, a remarkable increase of IAA-like promoters and a drastic decrease of ABA-like inhibitors were detected in the inoculated cuttings. Fungi- and IBA-induced effects were similar. According to Tomaszewski and Wojciechouwska (1973), it may be hypothesized that mycorrhizal fungi release some growth factors into the medium which interact with endogenous growth substances in the cuttings.

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PHOSPHATASE AND NITRATE REDUCTASE ACTIVITIES OF PISOLITHUS TINCTORIUS: INTRASPECIFIC VARIATION AND ECOLOGICAL INFERENCES

By

Iwan Ho and James M. Trappe

Keywords--mycorrhizal fungi, enzymes, organophosphorus, competition

Introduction

Isolates of Pisolithus tinctorius differ in culture characteristics and effectiveness as inocula in bareroot and container nurseries (Marx 1981, Molina 1979). To better understand such differences and some ecological characteristics of this fungus, we compared isolates from different regions and host associations for growth rate, acid and alkaline phosphatase and nitrate reductase activities, and acid phosphatase isozyme patterns.

Methods and Materials

Eight isolates (three each from Georgia and California and one each from Oregon and Washington) were obtained from sporocarps, four associated with Pinus spp., two with Psuedotsuga menziesii, one with Lithocarpus densiflora, and one with Quercus garrayana. For each analytical procedure, five replicate liquid cultures of each isolate were grown at room temperature in a completely randomized design. Analytical methods for acid and alkaline phosphatases, nitrate reductase, and acid phosphatase isozymes will be detailed in a future report. Results were subjected to analysis of variance.

Results and Discussion

Individual isolates differed significantly in growth rate, but the differences related neither to geographic source nor associated host. P. tinctorius showed acid and alkaline phosphatase and nitrate reductase activities weaker than have been demonstrated for other ectomycorrhizal fungi (Ho and Trappe 1980; Ho and Zak 1979; Ho, unpublished data). The phosphatase are crucial for utilization of organically bound phosphorus. Even though P. tinctorius can utilize a variety of carbon sources, its growth is relatively slow on all but glucose, mannose, and trehalose, even when "starter" glocose is available to enable activiation of adaptive enzymes (Lamb 1974). Thus, it seems to be weak in breaking down organic substrates.

P. tinctorius has proven especially effective as a symbiont of pines in harsh sites with low organic matter (Ruehle and Marx 1979). In forest soils of the Pacific Northwest, which typically abound in organic matter, P. tinctorius has promoted survival and growth of seedlings in plantations in only a few of many test sites (Castellano and Trappe, unpublished data). We hypothesize that its relatively poor capability to produce phosphatases limits its competitive ability where organophosphorus is a major phosphorus source. Production of enzymes for utilization of organophosphorus and nitrate nitrogen consumes both energy and nitrogen. Perhaps P. tinctorius particularly benefits its hosts in low organic soils by using host metabolites more for mycelial growth than for enzyme production. Mycelial exploration of a large volume of soil for readily available, mineralized nutrients could be advantageous in low organic soils. In high organic soils, fungi with higher phosphatase and decompositional capability might have the competitive advantage in obtaining nutrients.

Acid phosphatase isozymes of P. tinctorius separated readily in starch-gel electrophoresis. All isolates except one from California shared at least one allele, a greater homogeniety of acid phosphatase loci than we have found in other ectomycorrhizal fungi (Ho and Trappe, unpublished data). Phosphatase and nitrate reductase activities varied significantly between isolates, however.

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EFFECTS OF SEVERAL OSMOTICA ON THE GROWTH OF ECTOMYCORRHIZAL FUNGI IN LIQUID CULTURE

K. S. Diebolt and K. W. Mudge

This work is part of an ongoing project whose ultimate goal is to select fungal isolates capable of enhancing tree seedling survival and establishment on harsh sites. Our approach entails establishing the correlation between pure culture growth of ectomycorrhizal fungi under solute-induced osmotic stress and their ability to confer drought stress resistance to the host. The addition of solutes to nutrient solutions as a means of lowering their water potential has been used as a tool for studying drought stress in a wide range of organisms including mycorrhizal fungi (Mexal & Reid, 1974; Theodorou, 1978).

An ideal osmoticum should not be readily taken up by the fungus, not be readily metabolized, and it should be non-toxic. Based on these three criteria, polyethylene glycol (PEG) in the molecular weight range of 4000 to 6000 is frequently considered the osmoticum of choice. Based on preliminary experiments, we concluded that PEG 4000 was not a suitable osmoticum for these studies due mainly to poor oxygen diffusion. The present experiment was initiated to investigate the feasibility of using one or more low molecular weight solutes instead.

Materials and Methods

This experiment was designed to examine the effects of three osmotica each at four water potentials (-0.2, -1, -2, -3 MPa) on the growth of Rhizopogon vinicolor Smith and Pisolithus tinctorius Pers. (Coker and Couch). The basal nutrient medium was MMN with 10 g/L glucose.

A slurry of vegetative inoculum was prepared from mycelium previously grown on liquid MMN. One ml of slurry was added to each treatment flask containing 30 ml of nutrient solution. Fungi were grown in the dark at 24°C without agitation. At the end of five weeks and once a week through week eight, five replicate cultures were sampled from each treatment.

Results and Discussion

Figure 1A demonstrates that KCl is an unsatisfactory osmoticum since no growth occurred even at the lowest concentration (-1 MPa). This is surprising since there are several reports of the use of KCl with a wide range of non-mycorrhizal fungi (Luard, 1982) and mycorrhizal fungi (Theodorou, 1978). When grown on sorbitol, dry weight accumulation after eight weeks was greater for R. vinicolor than for P. tinctorius at all osmotic potentials except on basal medium (Figure 1-B). Note that the dry weight of R. vinicolor grown at -1 MPa was greater than in the basal MMN treatment. Growth of the two fungi relative to one another was reversed, however, when PEG 200 was used as the osmoticum (Figure 1-C).

The data shown in Table 1 suggest that none of these are ideal osmotica as each is metabolized to some extent by all three species of ectomycorrhizal fungi tested. Nevertheless, growth of Laccaria laccata (Scop. ex Fr.) Berk. & Br. and Cenococcum geophilum Fr. was less on PEG 200 than on sorbitol and about equal on PEG 200 in the case of P. tinctorius.

Conclusions

Based on the three criteria mentioned above, it appears that PEG 200 is a more suitable osmoticum than either sorbitol or KCl for screening fungi for tolerance of osmotic stress. (1) KCl is apparently toxic to both fungi tested; (2) variability is greater for fungi grown on sorbitol as compared to PEG 200; (3) metabolism of PEG 200 is somewhat less than that of sorbitol across several species of fungi. Experiments are in progress wherein the growth of drought stressed seedlings inoculated with ectomycorrhizal fungi will be evaluated. The relative performance of fungi grown in pure culture under osmotic stress induced by PEG or by sorbitol will then be compared to the performance of stressed seedlings inoculated with those fungi. It is the correlation between whole-plant/fungus studies and the data presented here that will be the ultimate test of which is the most "ideal" osmoticum.

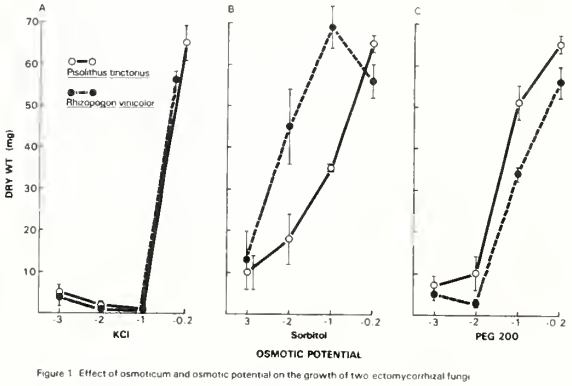

Figure 1. Effect of osmoticum and osmotic potential on the growth of two ectomycorrhizal fungi

TABLE 1. Growth of three ectomycorrhizal fungi on sorbitol or PEG 200 as sole carbon sources (all @ 10g/kg MMN)

Fungus	Percentage growth on Glucose: Sorbitol	PEG 200
Pisolithus tinctorius	16.3	20.4
Laccaria laccata	12.5	6.3
Cenococcum geophilum	48.8	23.25

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IS ETHYLENE INVOLVED IN ECTOMYCORRHIZAE FORMATION ON MUGO PINE?

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Introduction

Since a number of auxin effects in higher plants are mediated by auxin-stimulated ethylene production, Graham and Linderman (1980) have proposed that fungal-produced auxins may influence mycorrhizal development via ethylene. Using Douglas fir they have obtained evidence to support this hypothesis and they have demonstrated that pure cultures of ectomycorrhizal fungi are themselves capable of ethylene synthesis.

Mugo pine (Pinus mugo) was used in this study to test the same hypothesis. In the first part of this study, ethylene and auxin effects were investigated using isolated root organ cultures rather than intact plants. The effects of ethylene on mycorrhiza formation were tested on the whole plant level using an axenic mycorrhizal synthesis system.

Materials and Methods

Root Organ Cultures - Radicles from aseptically germinated Pinus mugo greater than 2 cm long were excised and placed in a root culture medium in Erlynmeyer flasks, and held in darkness at 24°C on a rotary shaker at 40 rpm. Root organ cultures were not clonal. The culture media was based on Slankis (1948) and contained 4% glucose in the case of experiments with ethephon and 7% sucrose in the case of experiments with auxin.

Axenic Seedling Culture - Aseptically germinated seedlings were placed in an axenic tube culture system similar to that of Yang and Wilcox (1983). The ectomycorrhizal fungi used to inoculate these seedlings was Pisolithus tinctorius Pers. (Coker and Couch), isolate T1069.

Results and Discussion

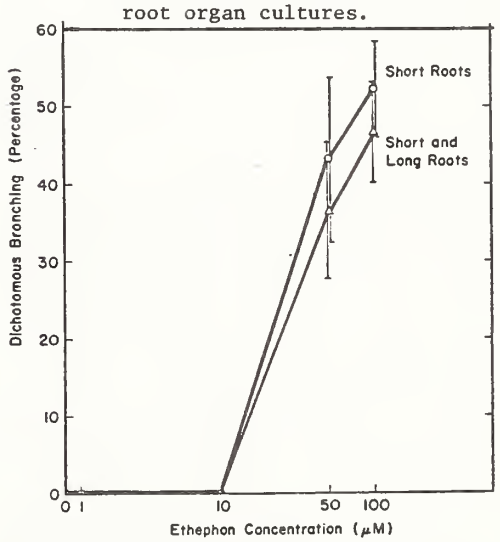
Development of control root organ cultures which were not treated with growth regulators (ethephon or auxin) was much the same as that of the root system of intact non-mycorrhizal plants, i.e., short roots were largely monopodial (less than 1% spontaneous bifurcation) and profusely covered with root hairs. Treatment with ethephon, an ethylene releasing compound, resulted in extensive dichotomous branching (a characteristic of pine mycorrhizae) at concentrations of 50 uM or greater (Figure 1). Frequently, several orders of dichotomy occurred on a single short root resulting in a coralloid structure. Parallel to the increase in dichotomous branching in response to ethephon was a marked decrease in root hair formation.

As has been reported previously (Slankis, 1975), when pine root organ cultures were treated with auxin (NAA) pinnate branching occurred as well as dichotomous branching. Dichotomous branching in response to NAA never exceeded a single order of bifurcation as was the case with ethephon. The optimal concentration for NAA stimulated dichotomous branching was 10 uM. NAA also stimulated ethylene production by mugo pine root organ cultures. That this auxin stimulated ethylene production may have been, at least in part, related to the effects of auxin on dichotomous branching is supported by the addition of the inhibitor of ethylene action, STS (100 uM), which resulted in a 34% reduction in NAA stimulated dichotomous branching. Also, in whole seedlings inoculated with P. tinctorius, dichotomous branching was stimulated by ethephon.

Dichotomous branching of pine short roots is a phenomenon distinctly different from lateral root formation. Thus the finding that ethylene stimulates dichotomous branching of pine root organ cultures represents an ethylene response not previously reported. Furthermore, the dramatic inhibition of root hair formation is in sharp contrast to numerous previous reports of ethylene stimulation root hair formation (Abeles, 1973).

The results of these experiments with isolated pine root organ cultures are consistent with the interpretation that the growth regulators, ethylene and auxin, may be involved in the morphological changes associated with ectomycorrhizae formation. The whole plant experiment in which ethylene stimulated dichotomous branching of the roots of pine seedlings inoculated with P. tinctorius is also consistent with that observation.

FIGURE 1. The effect of ethephon concentration on dichotomous branching of Mugo pine root organ cultures.



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^{13}C NMR STUDY OF MANNITOL AND ARGININE BIOSYNTHESIS DURING GLUCOSE UTILIZATION BY THE ECTOMYCORRHIZAL FUNGUS *Cenococcum graniforme*

by

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^{13}C nuclear magnetic resonance (NMR) spectroscopy has been used to follow the metabolism of (1 - ^{13}C) glucose in aerobic suspensions of the N-starved symbiotic ascomycete *Cenococcum graniforme*. The fate of ^{13}C label was analysed *in vivo* and in mycelia extract.

During the first day of incubation, the major metabolite produced from (1 - ^{13}C) glucose utilization was mannitol, the main storage carbohydrate of *C. graniforme*. From the observed isotopic enrichment in mannitol C1 it is concluded that the mannitol dehydrogenase pathway is the main route of mannitol formation. Glucose catabolism furnishes also a trehalose pool which is rapidly turning over, and it appears that apart from mannitol synthesis one of the main uses of glucose is the production of trehalose. The disaccharide presumably functions as a transitory storage carbohydrate. From the pattern of labeling of trehalose it is clear that a high proportion of the substrate glucose is converted to trehalose indirectly via the mannitol cycle. The contribution of this cycle was unexpected and it is proposed that its main role is to provide NADPH for ammonium assimilation and triglyceride synthesis.

Since the labeling pattern of arginine reflects directly the isotopic enrichments of glutamate and then α-ketoglutarate, ^{13}C NMR of arginine has been used to study the glucose catabolism. Biosynthesis from 99 % (1 - ^{13}C) glucose results in a nonrandom distribution of the ^{13}C label among the C1, C2, C3, C4 and C5 sites, with (2, 3, 4-^{13}C) arginine as the major product. A major biosynthetic pathway for synthesis of glutamate and then arginine from glucose was determined to be the Embden Meyerhof glycolytic pathway followed by the first third of the tricarboxylic acids cycle. This suggest that in N-starved mycelia, accumulation of arginine is due primarily to the reduced activity of α-ketoglutarate dehydrogenase which, in effect, shuts down the Krebs cycle. As a result α-ketoglutarate levels are elevated and, in the presence of ammonia, glutamate and then arginine levels will consequently become elevated.

Different metabolic pathways are associated with different correlations in the enrichment of the carbons, reflected in the spectrum as different ^{13}C - ^{13}C scalar multiplet intensities. Hence, intensity and ^{13}C - ^{13}C multiplet analysis allows quantitation of the pathways involved. The pathway suggested above is not the only route of arginine synthesis. The importance of other pathways, such as glyoxalate cycle and/or multiple turns of Krebs cycle, is demonstrated by deviations from the above pattern, e.g. labeling of C1, C2, C3, C5 of arginine, which also occurs to a significant extent.

It appears that glyoxylate cycle contributes for at least 30 % in the utilization of acetate.

Important enrichment of arginine C5 position corresponding to the glutamate C5 carboxyl, shows the existence of a futile cycle in which label from the C2 of acetate is "scrambled" to the acetate C1. By this way, phosphoenolpyruvate, formed from oxaloacetate by pyruvate carboxylase, returns to the Krebs cycle through pyruvate and acetyl-CoA.

Another interesting result is that the ^{13}C spectra show also the labeling at C6 guanidino position. This carbon derived directly from $H^{13}CO_3$ produced by decarboxylation of (1 - ^{13}C) glucose through hexose monophosphate shunt and decarboxylation of Krebs cycle intermediates. The enrichment allows thus an estimation of the carbamoyl phosphate synthetase activity.

The use of ^{13}C NMR has provided a comprehensive insight into several aspects of the carbon metabolism of the ectomycorrhizal fungus *Cenococcum graniforme*. A main result has been that the tricarboxylic acid cycle furnishes a high intracellular pool of arginine, and it appears that apart from mannitol synthesis one of the main uses of glucose and acetate is the formation of arginine and trehalose.

GLUTAMINE SYNTHETASE/GLUTAMATE SYNTHASE PATHWAY FOR AMMONIUM ASSIMILATION IN BEECH ECTOMYCORRHIZAS

by

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Keywords--Fagus sylvatica, Lactarius sp., Russula sp., nitrogen, glutamate dehydrogenase.

Introduction

The few studies carried out on the nitrogen metabolism in ectomycorrhizas have suggested an important role for the fungal partner. However, the nitrogen assimilation pathway (s) has not been elucidated.

Both the GS/GOGAT* pathway and the GDH pathway of N assimilation were potentially operative in the ectomycorrhizas containing both fungal and higher plant cells.

The work reported here was designed to elucidate the pathway of ammonia assimilation in beech ectomycorrhizas. Three approaches were used including (a) amino acids evolution after ammonia feeding, (b) a time course study following the metabolism of absorbed 15N-ammonia to amino acids, (c) an investigation of the effect of the inhibitors methionine sulfoximine (MSX) and albizin (ALB).

Material and methods

1. Plant material. Fagus sylvatica roots with Lactarius sp. and Russula sp. ectomycorrhizas were collected from the upper humic layers (AO) in a beech stand in the Vosges (acid brown soil) with a moder humus.

2. NH_4^+ and 15N feeding procedure. Whole detached ectomycorrhizas were placed in an aerated flask containing 100 mM glucose, 7 mM KH_2PO_4 2,5 mM $MgSO_4$ and 5 mM $(NH_4)_2SO_4$ in 0,5 l (pH 5.5). In addition, streptomycine (0,04 %) and tifomycine (0,4 %) were added to prevent bacterial growth.

When needed, the ammonium feeding solution was replaced by one containing 15N-ammonium (50 atom % excess). L-methionine-DL-sulfoximine (MSX) and albizin (ALB) were added to the ammonium feeding solution at a concentration of 5 and 2.5 mM, respectively.

3. Analytical procedures. Amino acids analysis were carried out by high performance liquid chromatography. Determination of 15N-amino acid enrichments was performed by using gas chromatograph-mass spectrometer.

Results

The absorption of nitrogen by beech ectomycorrhizas is associated with synthesis of large amounts of glutamine and arginine. This indicates that the newly absorbed nitrogen was rapidly assimilated. Glutamine pattern reflects its key position in the initial steps of ammonium assimilation. During the first two hours of labeling, the 15N enrichment of the free amino acids remained at a low level and then, it increased sharply. The major recipients of newly assimilated 15N were glutamine, glutamate, γ-aminobutyrate and alanine. The labeling of these metabolites were similar and appeared to become saturated at around 25 to 30 % 15N atom excess indicating that "storage pools" of these compounds exist in the ectomycorrhizas. The other amino acids showed lower enrichment, but this increased rapidly with time, especially asparagine.

When MSX was included as a GS inhibitor, no enrichment was found in glutamine. In addition, the incorporation of newly absorbed labeled nitrogen into glutamate and other free amino acids have dramatically decreased. The amount of labeled nitrogen incorporated into glutamine and other amino acids in the MSX-treated ectomycorrhizas were between 0 and 12 % of the amount detected in the control symbiotic tissues. The small incorporation of label into glutamate and derivatives (alanine, γ-aminobutyrate and aspartate) could be accounted for a low GDH activity or a residual GS activity. The rate of assimilation via GDH is therefore considered to be no more than 10 % of the total assimilation.

When ALB which inhibits all glutamine amide transfer reactions was present in the incubation medium a similar pattern was obtained. Label in glutamine, glutamate and other amino acids remained at a quite low 15N atoms % value.

Both inhibitors greatly reduced the 15N recovered in alanine, γ-aminobutyrate and other amino acids and therefore, demonstrate the need for glutamate and glutamine as nitrogen donors.

Discussion

The evidence based on 15N labeling of amino acids and the effects of the GS/GOGAT pathway inhibitors is consistent with the GS/GOGAT pathway of ammonia assimilation being the major route of ammonia assimilation in beech ectomycorrhizas. It appears that, despite the high ammonia concentration used in the present study, the GDH pathway does not play a substantial role in the assimilation of ammonia in ectomycorrhizas.

Except certain genetic mutants of Neurospora crassa, the fungi use GDH as a major route for ammonia assimilation. We have recently shown that this pathway is also solely responsible for the ammonia assimilation in the ectomycorrhizal C. graniforme.

Therefore, if Lactarius sp. and Russula sp. behave as the other filamentous fungi, the importance of the GS/GOGAT pathway in ectomycorrhizas suggests that the fungal partner plays a little role in the primary incorporation of nitrogen into organic compounds.

* GDH : Glutamate dehydrogenase ; GS: Glutamine synthetase ; GOGAT : glutamate synthase

COMPATIBLE AND INCOMPATIBLE ECTOMYCORRHIZAL HOSTS OF SUILLUS GREVILLEI

By

J.A. Duddridge

Keywords--Larix kaempferi, Pinus sylvestris, Pseudotsuga menziesii, Betula pubescens, ultrastructure, host - fungus interface, host specificity

Introduction

Suillus grevillei (Klotzsch) Sing. is an ectomycorrhizal symbiont that has been reported to be highly specific for Larix spp, although there is evidence to suggest that it is not as specific as was originally thought. (Trappe, 1962; Linneman, 1971; Molina and Trappe, 1982). A comparative ultrastructural study has been carried out to look at the host-fungus interface formed in interactions between S. grevillei and a number of ectomycorrhizal hosts and to determine whether the presence of glucose in the synthesis medium has any effect on the development of the interface.

Methods and Materials

Sterile petri dishes were filled with a 1:4 mixture of peat and vermiculite moistened with a 1/10 dilution of modified Melin-Norkran solution (5:4 w/v) in the presence and absence of glucose. Each petri dish was inoculated with an agar plug of S. grevillei. An aseptically germinated seedling of one of the folllowing: Larix kaempferi(Lamb.) Carr., Larix decidua Mill., Pinus sylvestris L., Betula pubescens Ehrh., Pseudotsuga menziesii (Mirb.) Franco, Pinus nigra Arnold, Picea sitchensis (Bong.) Carr, Picea abies (L.) Karst. or Alnus glutinosa (L.) Gaertn. was introduced through a notch cut in the petri dish which was sealed with sterile vaseline and plastic tape. The petri dishes were placed in a controlled environment growth cabinet (temperature 15° day, 10° C night; light: 120μ E $m^2 s^{-1}$, 16 hour day, 8 hour night cycle). Root samples were taken weekly and prepared for TEM and SEM as described previously (Duddridge and Read, 1982).

Results and Discussion

In the absence of glucose ectomycorrhizas were synthesised with L. kaempferi, L. decidua, P. sylvestris and P. menziesii but not with B. pubescens, P. nigra, P. sitchensis, P. abies and A. glutinosa, confirming that although S. grevillei has a narrow host range it is not restricted to Larix spp. Transmission electromicrographs of the host-fungus interface are shown in Figures 1 and 2 respectively.

When glucose was included in the synthesis medium a rudimentary sheath was formed round the roots of B. pubescens and callose-like wall appositions (WA) were found on the inside of the host cell adjacent to points of contact with the hyphae. The host cell* appeared dead. In interactions between S. grevillei and P. sylvestris intracellular hyphae were frequently found inside living host cells and were surrounded by a callose-like encasement material (EM). Alterations were observed in the cell wall adjacent to both the Hartig net** and sheath. In mycorrhizas synthesised between S. grevillei and L. kaempferi in the presence of glucose, the host cell wall adjacent to the sheath was thicker and contained electron-dense deposits. These changes in the host-fungus interface of synthesised mycorrhizas may be the result of a defence reaction on the part of the host to the increasing dominance of the fungal partner due to high external levels of carbon.

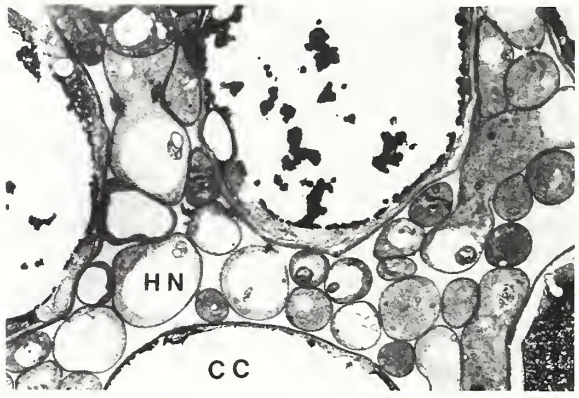

Figure 1. Suillus grevillei + Larix kaempferi ectomycorrhiza. Mag. x 3,125

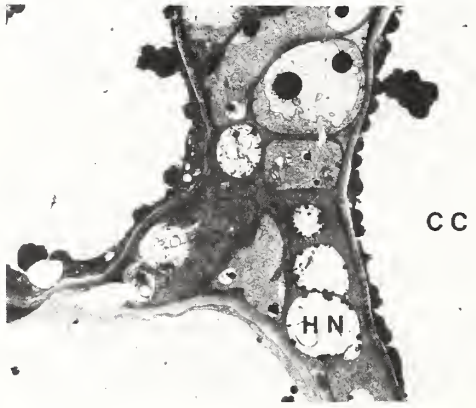

Figure 2. Suillus grevillei + Pseudotsuga menziesii ectomycorrhiza. Mag.x 3,125

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* HC; ** HN

BASIDIOSPORE GERMINATION IN THE RHIZOSPHERE

By

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Keywords--Pinus radiata, Rhizopogon, specificity, root exudates

Introduction

Germination of basidiospores of ectomycorrhizal fungi in laboratory media rarely exceeds 0.1% even in the presence of activator organisms, but Fries and Birraux found that laying pine roots on agar media seeded with Hebeloma (Agaricales) spores increased germination to 1%. Our studies show exudates from pine roots stimulate germination of Rhizopogon (Hymenogastrales) to 60% and that this is specific to pines.

Methods and Materials

The method was described (with bacteria) by Bowen[3]. Spores of Rhizopogon luteolus (mycorrhizal on Pinus radiata)were suspended in 1% water agar at 36°C. Roots (6 cm long) sterile P.radiata seedlings (21 days old) were dipped into this and drained. This inoculated the roots uniformly with a thin layer (a few microns thick) of spores suspended in agar.

Roots were then placed in sterile vermiculite at 25°/15°C day/night temperature, removed at intervals, stained with Jones and Mollison[4] stain and examined for spore germination. Counts were made of 40 microscope fields at the base, mid-root and apex.

Results

(a) After 18 days, 65% ± 13% of spores germinated in the basal parts of the root, 39 ± 21% in the mid-root and 14 ± 17% in the apical cm.

Bacterial colonies also developed in the rhizosphere. These did not stimulate germination in agar in the absence of roots.

(b) Germinating spores were removed to agar and single colonies were used to inoculate P.radiata in sterile soil. These formed typical ectomycorrhizas.

(c) The experiment was repeated with 3 day-old seedlings of P.radiata, Eucalyptus globulus, Medicago truncatula, Trifolium subterraneum, Lolium perenne, and sterile glass fibres. After 5 weeks basidiospores had germinated only in the rhizosphere of P.radiata.

Conclusion

We conclude that Pinus radiata produces specific substances which may not occur in non-host plants and which lead to high levels of basidiospore germination. Similar specificity in stimulating basidiospore germination was shown by Birraux and Fries[2]. The high germination is consistent with that predicted by dose - mycorrhizal infection curves with basidiospores of R.luteolus [5].

Current studies are examining root exudates and the effects of nutrition factors on germination of basidiospores in the rhizosphere.

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SPORE GERMINATION IN ECTOMYCORRHIZAL FUNGI

By

Nils Fries

Keywords: Rhodotorula, Hebeloma, Thelephora, Laccaria, Lactarius, Leccinum, tree root exudate

Introduction

The spores of ectomycorrhizal basidiomycetes, mainly agarics and boleti, are notorious for their unwillingness to germinate in vitro. In recent years several studies have been performed to find conditions permitting germination of these fungi. This paper gives a brief summary of the progress made in this field by several different workers.

Materials and Methods

The results to be reported are generally based on experiments with sterilized nutrient agar plates in Petri dishes. The spores are dispersed on the surface of the plate, where they and their germination can be studied under the light-microscope. To some extent also experiments have been made with hanging-drop cultures and with spores introduced in the rhizosphere of axenically grown tree seedlings.

No nutrient medium has been found which permits germinations in all, or not even most, ecto-mycorrhizal basidiomycetes. In most of the successful cases it appeared that the presence of another living organism together with the spores was essential for germination. However, certain further, particular circumstances also need to be considered if germination shall occur, at least in some taxonomic groups:

(1) The content of ammonium must be kept low, since this ion inhibits germination in many species;
(2) An inhibitory agarose product, formed during autoclaving, must be eliminated, preferably by adding activated charcoal powder to the medium;
(3) In some species natural products, like peptone or malt extract, added to the medium improve germination;
(4) In many species germination occurs only after an incubation time of several weeks;
(5) Spore collections, even if stored at low temperatures, may lose their germinability as soon as within a month or two.

Results and Discussion

In all cases of induced spore germination in these fungi one or another of the following modes of induction proved successful.

(1) Germination without an activator organism is rare. It has been observed in some species of Tricholoma, Hebeloma, Suillus, and a few other genera. Germination is always sparse and slow.

(2) Tree roots induce germination in Hebeloma and Thelephora on agar plates, and in Laccaria, Pisolithus, Rhizopogon, and Scleroderma, perhaps also in Russula, when the spores are introduced in flask cultures with tree seedlings.

(3) Colonies of yeast, particularly Rhodotorula species, have long been known to induce germination, although slowly, in spores of numerous species in various genera, e.g., Boletus, Suillus, Laccaria, Paxillus, Amanita, Lactarius, and Cantharellus.

(4) In a similarly unspecific way some filamentous fungi trigger germination in certain genera, e.g. Laccaria, Suillus, and Thelephora. Ceratocystis fagacearum promotes germination in Lactarius by a volatile mycelial product.

(5) The most efficient germination inducing mechanism so far found in ectomycorrhizal fungi is that operating in Leccinum. The species of this genus distribute themselves in two groups, the aurantiacum and the scabrum group. In each group an exudate from any mycelium belonging to the group induces germination in any species of the group, but not in any other spores. Thus, there is a strict species-group specificity. Germination starts after a few days and at a high percentage. Homing of hyphae towards germinating spores takes place within and between species. Only in intraspecific homing will the two partners in the plasmogamy survive; the outcome of interspecific homings is always lethal.

All these interactions are controlled by numerous different substances exuded from roots, hyphae or spores. To chemically identify these substances and to elucidate biochemically their modes of action represents a large field of future research. The ecological implications of these studies are obvious.

References

Citations could not be included because of lack of space. References to all authors behind the statements in this paper can be found in a more comprehensive article on "Spore germination in the higher Basidiomycetes" in Proc. Indian Acad. Sci., Plant Sciences (in press).

THE ROLE OF ELICITORS IN ECTOMYCORRHIZAL FORMATION

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INTRODUCTION

Conifer and ectomycorrhizal fungi have coevolved to establish specific mutualistic interactions. Specificity may reside in recognition events which trigger a resistant response in incompatible hosts but not compatible conifer roots. Studies with plant pathogenic fungi suggest a resistant response, hypersensitivity, may be initiated by recognition of fungal components termed elicitors. Elicitor recognition in a compatible interaction may be circumvented by the production of additional fungal components, suppressors (4, 8).

We propose elicitors may function in the specificity of ectomycorrhizal formation. The system studied involves the fungi Rhizopogon occidentalis and R. vinicolor which form ectomycorrhizae with lodgepole pine and Douglas-fir respectively (7). Our approach has centered on seeking elicitor activity in extracellular fractions produced by the Rhizopogon species.

METHODS AND MATERIALS

Fungi were grown in agitated liquid culture in modified Melin-Norkrans media to middle log growth phase. Culture filtrates were concentrated by rotary evaporation and lyophilization prior to dialysis to remove components of molecular size less than 10 KD. Molecules in the dialysate were fractionated further by molecular size by Sepharose 6 B gel filtration. Ion exchange chromatography with DEAE Sephadex and CM Sephadex was performed. Protein and neutral sugar concentrations were determined in the eluates by colorimetric analyses (2, 5). Neutral sugar compositions were quantified by gas chromatography of alditol acetate derivatives (2).

Elicitor activity in the eluates was determined by treatment of cut surfaces of Dark Red Kidney bean cotyledons or intact callus initiated from Douglas-fir roots. Control tissues were treated with water. The assay was scored as positive if browning developed. The formation of soluble phenolic materials in treated tissues was demonstrated by use of Folin reagent (5), and measuring absorbance at 280nm of ethanolic extracts.

RESULTS AND DISCUSSION

Culture filtrates from both Rhizopogon species contained components active as elicitors on Dark Red Kidney bean cotyledons and Douglas-fir tissues. The production of brown oxidized phenolics was detected as soon as 12 hours after application of fungal material containing 5µg glucose equivalents/ml to the plant tissues.

Chromatography of the extracellular Rhizopogon products on Sepharose 6 B yielded fractions of three sizes: greater than 90 KD; intermediate between 60 and 90 KD and less than 60 KD. Each of these three fractions possessed elicitor activity but displayed different neutral sugar compositions (Table 1). The compositions of fractions obtained from R. vinicolor differed from those of R. occidentalis. Similar differences in carbohydrate compositions have been implicated in determining the specificity of recognition events for other systems (1).

Ion exchange chromatography of the Rhizopogon extracellular materials produced non-adsorbed fractions that were carbohydrate rich and adsorbed glycoproteins. Because the adsorbed and non-adsorbed moieties demonstrated elicitor activity, it is possible that the Rhizopogon species produce more than one type of elicitor detectable by conifer, a potential host tissue. Characterization of the Rhizopogon elicitors will determine whether they resemble the fungal wall structural components (glucans, chitin and unsaturated lipids) that are reported to have elicitor activity in other plants (4, 8). Alternatively elicitor activity may depend on an enzymic function, a pectin-degrading activity (3, 6).

The current elicitor preparations do not display specificity on the Douglas-fir callus. Future studies will indicate whether the elicitors have species specific structure and whether suppressor components are produced. The production of elicitors by ectomycorrhizal fungi raises the question of the role of such structures in the plant-symbiont interaction. Understanding elicitor function may promote the utilization of mycorrhizal fungi in reforestration and reclamation projects.

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TABLE 1

THE NEUTRAL COMPOSITION OF EXTRACELLULAR PRODUCTS FROM RHIZOPOGON OCCIDENTALIS AND R. VINICOLOR

Molecular Size[a] of fraction	% Neutral Sugar Composition[b] Fucose	Xylose	Mannose	Galactose	Glucose
R. occidentalis					
> 90 KD	1(±1)	19(±2)	52(±5)	11(±8)	17(±3)
60-90 KD	9(±3)	5(±2)	27(±4)	34(±5)	26(±3)
< 60 KD	0	2(±2)	21(±6)	3(±2)	75(±1)
R. vinicolor					
> 90 KD	0	3(±2)	40(±6)	28(±9)	29(±1)
60-90 KD	2(±1)	0	22(±2)	37(±9)	38(±9)
< 60 KD	0	0	22(±1)	26(±10)	50(±12)

[a] Culture filtrates products were fractionated into molecules of size greater than 90 KD, between 60-90 KD and less than 60 KD by Sepharose 6 B chromatography.

[b] The sugar compositions of the Rhizopogon fractions were determined by alditol acetate analysis.

FIGURE 1

Culture filtrate was applied to DEAE Sephadex, eluted with 20 mM Tris-HCl pH 8.2 and collected as 5 ml fractions, numbers 1 to 20. A gradient of 0 to 1.0M KCl in 20 mM Tris-HCl pH 8.2 was applied, fractions 30-60. 1.0M KCl in 20 mM Tris-HCl pH 8.2 eluted fractions 60-120. Each fraction was assayed colorimetrically for carbohydrate and protein (2, 5).

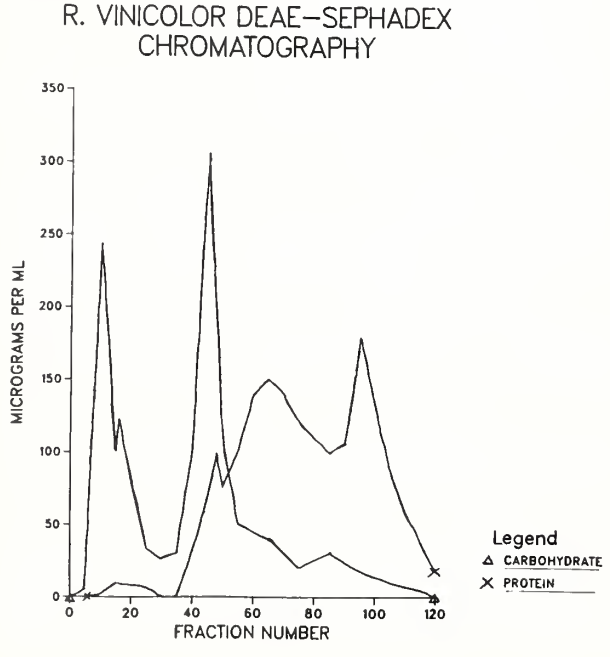
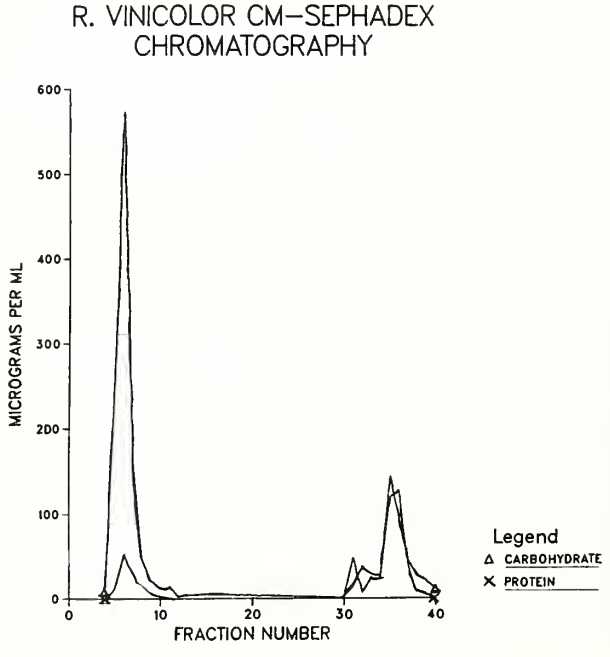


FIGURE 2

Fractions 40-60 from DEAE Sephadex were applied to CM Sephadex and eluted in 5 ml fractions (1 to 15) with 20 mM potassium acetate pH 5.0. Fractions 16 to 40 were eluted with 20 mM potassium acetate pH 5-1.0M KCl. Each fraction was assayed colorimetrically for protein and carbohydrate (2, 5).



GROWTH OF MYCORRHIZAL BIRCH IN ELEVATED LEVELS OF COPPER AND NICKEL

By

Melanie D. Jones and Thomas C. Hutchinson

Keywords--Betula papyrifera, tolerance, heavy metals, Laccaria, Lactarius, Scleroderma

Introduction

The role of mycorrhizae in host metal tolerance varies. While ericoid mycorrhizae are essential for host growth under high Cu and Zn conditions (Bradley et al. 1982), VA mycorrhizae may cause increased uptake of toxic metals (Killham and Firestone 1983). The objective of this study was to determine if Betula papyrifera seedlings inoculated with different ectomycorrhizal fungi differ from each other and from nonmycorrhizal seedlings in their ability to grow in the presence of elevated levels of Cu or Ni. The effect of infection on uptake of the metals was also evaluated.

Methods

Sterile Betula papyrifera Marsh. seedlings, growing in silica sand in 50 ml culture tubes, were inoculated with either Laccaria proxima, Lactarius hibbardae, L. rufus, Scleroderma flavidum, or not inoculated. Following infection seedlings were transferred to pots and watered every 2 weeks with 1/10 modified Ingestad's solution with 4ppm Cu, 5ppm Ni, or no metal added. After 18 weeks, seedlings were digested and the digests analyzed for Cu or Ni.

Results and Discussion

Cu treatment reduced seedling weights to approximately 40% of control weights. Mycorrhizal seedlings weighed an average of 30% less than the nonmycorrhizal seedlings (orthogonal comparison, $p<0.001$). Growth differences between fungal treatments were also present (L. proxima 71.3 g, L. hibbardae 88.9 g, L. rufus 51.8 g, S. flavidum 96.5 g, $p<0.001$). While root weights differed with fungal treatment shoot weight was the component of growth most reduced in L. rufus seedlings.

While total seedling weight was inversely correlated with stem Cu concentration ($r=0.60$, $p<0.01$), differences in Cu tolerance between treatments could not be explained by differences in Cu uptake. Cu content of roots was directly related to root weight (i.e., root concentrations did not differ). Stem Cu contents were not significantly affected by fungal treatment.

Ni-treated seedlings weighed approximately 63% of non-metal controls. While on average, weights of the mycorrhizal and non-mycorrhizal seedlings did not differ (orthogonal comparison), differences occurred between fungal treatments (orthogonal comparison, $p<0.001$, Figure 1). Root, but not shoot weights differed between treatments ($p<0.001$, ANOVA).

With Ni, metal content again appeared to be positively related to root weight (Figure 2). Thus S. flavidum seedlings had much higher Ni contents than the other treatments. Unlike with Cu however, stem Ni contents varied with fungal treatment, and appeared to be inversely related to root Ni content. Thus, with the Ni-treated seedlings, good growth was associated with high root and low shoot Ni content. This may be explained by Ni retention by the fungus.

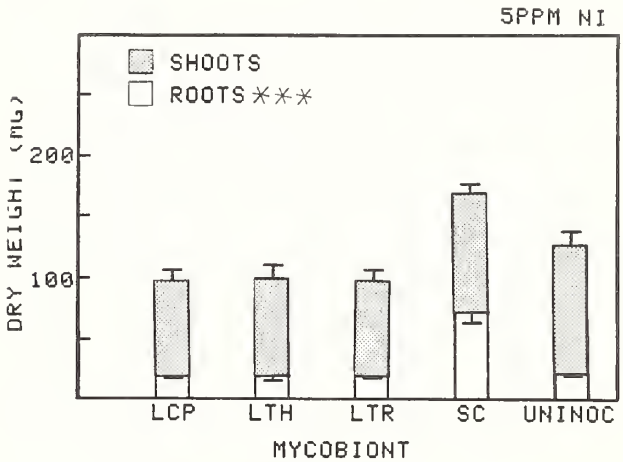


Figure 1. Dry weights of birch seedlings treated with 5ppm Ni. means ± s.e.'s *** $p<0.001$ for a one way ANOVA.

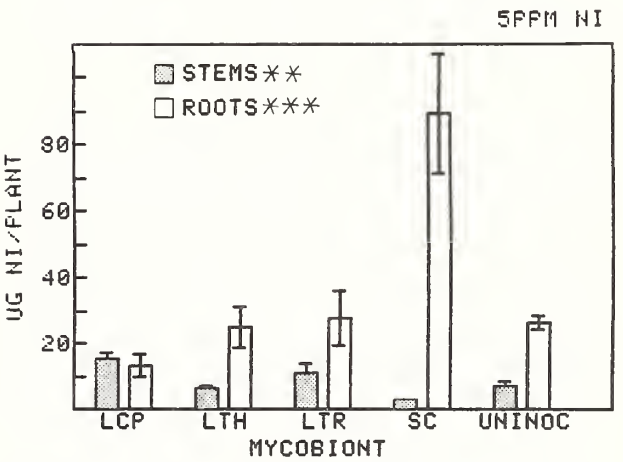


Figure 2. Ni content of birch seedlings grown in 5ppm Ni. Means ± s.e.'s, ** $p<0.01$, *** $p<0.001$ for a one way ANOVA.

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SPECIFICITY OF DIPTEROCARP MYCORRHIZA.

By

W.Th.M. Smits [+]

Keywords--Anisoptera marginata, ectomycorrhiza, host specificity.

Introduction

Dipterocarpaceae are a family of tropical hardwood species, mainly confined to south-east Asia. Their wood is known as meranti, keruing, bangkirai and kapur and makes up about 25% of the world trade in hardwood. Regeneration of dipterocarps in logged-over forest is problematic. To enrich logged-over forest with dipterocarp planting stock ectomycorrhiza were found to be of extreme importance (Smits, 1983).

Methods and Materials

Seeds and seedlings of different Dipterocarpaceae were collected in south-east Asia and grown in a greenhouse of the department of Silviculture of the Agricultural University Wageningen, in the Netherlands. From these seedlings we took many cuttings (Smits, 1983a).

Non-mycorrhizal plants were either inoculated with pure-culture grown mycelium or ectomycorrhizal roots, or were planted in special root boxes (Bosch, 1984) together with mycorrhizal dipterocarp plants (Fig.1.).

Results and Discussion

From our observations so far it seems that dipterocarp species to a certain degree have a specific preference for particular mycorrhizal fungi. Anisoptera marginata could not be infected by mycorrhizal roots of other dipterocarp genera. Neither could we establish mycorrhiza on very poorly growing non-mycorrhizal dipterocarp plants with general ectomycorrhizal fungi as Pisolithus tinctorius and Cenococcum geophilum. Non-mycorrhizal dipterocarps always show stunted growth and chlorotic leaves. Inoculation with the right fungus results in dramatic growth responses (Fig.2.). An obligate specific mycorrhizal relationship of dipterocarps can explain many features of the behaviour of dipterocarps in logged-over forest (Smits,1983).

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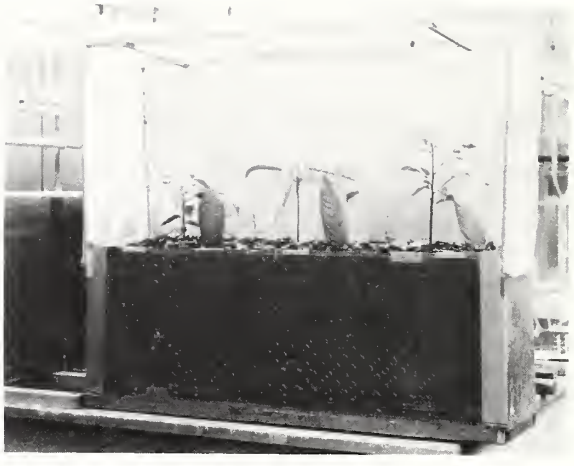

Figure 1.: Mycorrhizal and non-mycorrhizal dipterocarps growing in a "root box", that allows observation of the mycorrhizal development in the soil.

Figure 2.: Anisoptera marginata. Right: non-mycorrhizal seedling at age 4 years. Left: inoculated stem-cutting at age 1½ year.Average height difference is more than one meter.

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NITROGEN USE BY SPRUCE ECTOMYCORRHIZAS

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Keywords - lysimeters, mineralisation.

Introduction

The suggestion that profuse development of ectomycorrhizas and associated hyphae in the surface organic horizons of forest soils provides a mechanism for rapid and efficient exploitation of available mineral nitrogen is examined.

Methods

The data come from the forest floor of 37 year old Sitka spruce. Each month the following were estimated in three 0.04 ha plots.

1. Mineral N output in leachate from 3 zero-tension lysimeters installed at the junction of organic and mineral horizons.
2. Volume of, and mineral N input in, throughfall from 3 gauges.
3. Net N mineralisation in three 40 mm diam. intact cores wrapped in plastic film and incubated in situ.
4. Exchangeable mineral N content in three 40 mm diam. cores.
5. Number of live root tips in twelve 40 mm diam cores.
6. Fluorescein diacetate (FDA)-active hyphal length in 6 bulked 40 mm diam. cores.

Uptake and Inflow

Uptake into the root system can be estimated as shown below (for January 1983). In root-excluded areas exchangeable mineral N increases by an amount ± equivalent to the input in throughfall and mineralisation (Harmer & Alexander, 1984).

A.	Input in throughfall	0.497 kg N ha^{-1}
B.	Input from mineralisation	1.250 kg N ha^{-1}
C.	Change in exchangeable	+ 0.920 kg N ha^{-1}
D.	Output in lysimeter	0.118 kg N ha^{-1}
E.	Uptake (A+B-C-D)	0.709 kg N ha^{-1}

Inflow rate of N per root tip can be calculated as

$$\text{Inflow} = \frac{\text{N uptake} \times \text{Ln} \frac{(T_2)}{(T_1)}}{\text{Uptake period} \times (T_2 - T_1)} \quad \mu g \text{ N tip}^{-1} \text{ day}^{-1}$$

where T_1 and T_2 are the number of tips at the beginning and end of the uptake period.

Results

Monthly means are shown in Fig. 1. Peaks of uptake and inflow correspond to periods of heavy rainfall in May and September. Hyphal lengths also follow the pattern of throughfall but there is a lag before root tip numbers increase after the autumn rains. Total mineral N uptake for the year was 23 kg ha^{-1}, about 30% of the annual requirement for this stand. The efficiency of exploitation of available mineral N $\left(\frac{100.E}{A+B-C}\right)$ ranged from 68-100%, and did not drop below 90% from May to November.

Discussion

Uptake appears to depend upon availability rather

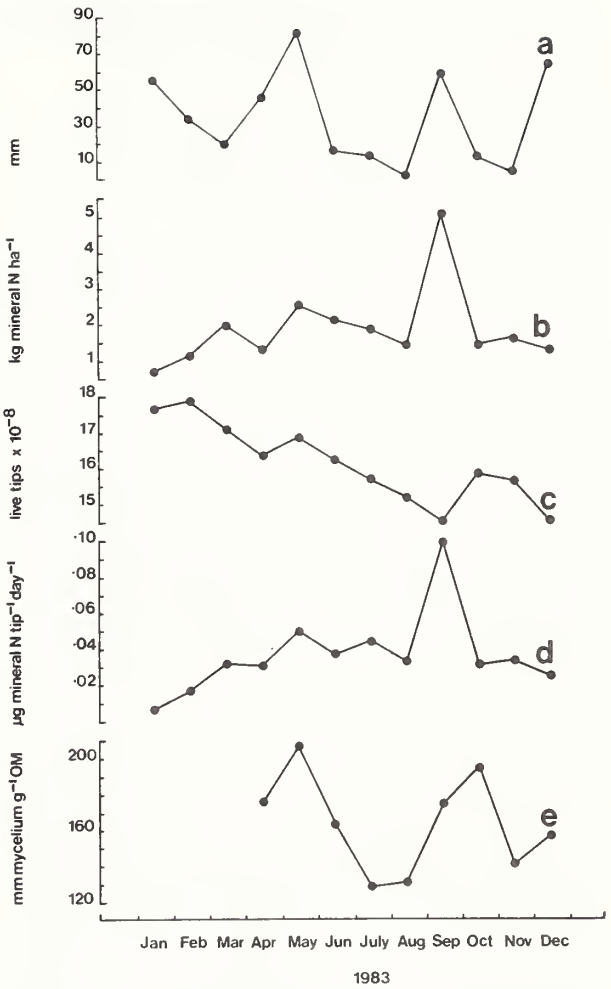

Figure 1. Seasonal trends in a) volume of throughfall, b) mineral N uptake, c) number of live root tips, d) mineral N inflow and e) FDA-active hyphal lengths. All data from the forest floor of a 37 year old spruce plantation.

than the number of root tips present and even the lowest number recorded is adequate to completely deplete the supply of mineral N. The response of hyphae to increased availability of water and N is more pronounced than that of the root tips: it would be interesting to know the proportion of these hyphae which are mycorrhizal. Inflow rates ranged from 0.007-0.103 µg N per tip per day. These compare with rates of from 0.009-0.780 µg N per tip per day found by Fairley (1983) in a 40 week pot experiment over a range of nitrogen regimes.

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SOME STUDIES ON *PISOLITHUS TINCTORIUS* IN *VITRO* AND *IN VIVO* AS INFLUENCED BY CAPTAN AND BRASSICOL (PCNB)

By

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Keywords - *Pinus patula*, Pure culture synthesis, growth stimulation, inhibition

Introduction

Fungicides like captan (N-trichloromethyl-thio-4 cyclohexene-1, 2-dicarboximide and brassicol (Pentachloronitrobenzene, PCNB) are widely used for control of seedling diseases. In an attempt to ascertain whether these fungicides have a direct effect on mycorrhizal fungus, laboratory culture of *Pisolithus tinctorius* (Pers.) Coker and Couch was tested with each of the fungicide. The study was extended to pure culture synthesis test of the fungus with *Pinus patula*.

Methods and Materials

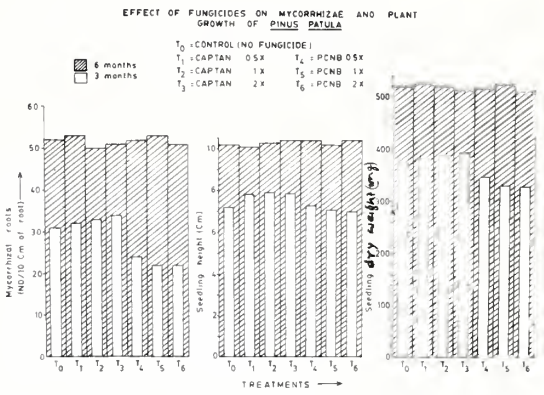
Modified Melin-Norkrans (MMN) agar medium (Marx, 1969) with glucose was prepared and autoclaved. Technical grade fungicides of the appropriate concentrations viz. single (1x), double (2x) and half the single (0.5x) application rates used in nursery practice were incorporated and poured into petri dishes. Twenty one days grown, 8 mm diameter of the test fungus *Pisolithus tinctorius* (obtained from Dr. D.H. Marx, USA), were seeded with each of the above petri dishes. Diameters of mycelial colonies were measured.

Liquid culture tests were made in 100 ml of autoclaved MMN medium in 250 ml conical flasks. Fungicides were incorporated as above and the flasks were seeded with test fungus. The mycelium were harvested and oven dry weights estimated.

The pure culture synthesis techniques of Molina and Palmer (1982) were adopted using glass-synthesis tubes. Test fungus, grown for 3 weeks was inoculated. One set of experiment was conducted without fungus inoculation. Fungicides were incorporated to the tubes and the seedlings were grown in air-conditioned plant culture room, with artificial light for 16 hrs. The seedlings were examined for their mycorrhizal short roots, height and dry weight.

Results and Discussion

Captan stimulated the test fungus in vitro as well as the axenic growth with *Pinus patula*. However, PCNB inhibited the mycorrhizal fungus in both cases (Fig). Trappe et al (1984) indicated that the dicarboximides tend to have no effect or even favour mycorrhiza formation. Marx and Rowan (1981) suggested that captan stimulated ectomycorrhizae development formed by specific non-target fungi. Studies of the effect of pesticides on the growth of ectomycorrhizal fungi in axenic culture indicate that different fungi can respond quite differently to a given chemical. The extrapolation of in vitro and pure culture studies to natural condition, may not give real situation as it is likely to be influenced by several unknown factors. In view of this, further studies on the effect of these fungicides in the nurseries are being planned.



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SOIL AND FUNGICIDE EFFECTS ON ECTOMYCORRHIZAL INOCULANTS AND SEEDLING GROWTH

By

JM Theron

Keywords – – Pinus radiata, Pisolithus tinctorius, Rhizopogon vulgaris

Introduction

This study is the initiation of a project to select ectomycorrhizae isolates which will consistently stimulate host growth appreciably over a relatively wide range of conditions.

Methods and materials

A pot trial with a 2^4+2+2 factorial design with 3 replications and 4 seedlings per pot was laid out to test the interaction between soil type (2), mycorrhizal fungi (2), inoculum type (2) and fungicide (2).

The 1ℓ pots were lined with polythene inserts, filled with steam-sterilized soil (sand or clay) and fitted with capped glass watering tubes. One Pisolithus tinctorius (Pt) and one Rhizopogon vulgaris (Rv) isolate, both ex Stellenbosch, were used as inoculants. Inoculum treatments (equivalent to 1ℓ vegetative inoculum or 5g spores in a mulch per m² pot surface) and Kaptan fungicide (equivalent to 200mg a.i. per m²) were applied in four positions and two Pinus radiata half-sib seeds were sown on top of the inoculum. Between sowing and germination, pots were covered by plastic bags to reduce competition to the test fungi by naturally occurring ectomycorrhizal air-borne spores. The seedlings were thinned to four per pot. A 2cm thick layer of sterilized chipped stone was spread over the surface of the pots. The pots were placed in a growth cabinet at 30/25°C, 16/8 hours day/night and 85% relative humidity. Sterilized water was applied via the glass tubes to return pots to field capacity once per week. This method of watering and the dry chipped stone mulch minimised the movement of air-borne spores into the pots.

After 7 months, shoot length, collar diameter, fresh mass and % survival were determined and ectomycorrhizal development visually estimated on each seedling. Representative ecto-mycorrhizae were sectioned, mounted in phloxinelactophenol, and examined for fungal mantle and Hartig-net characteristics at 100 x. From each isolate 100 surface-sterilized ectomycorrhizae were plated on MMN agar medium and incubated at 25°C for 4 weeks.

Results and Discussion

Table 1 summarises seedling parameters in the pot trial. Collar diameter and % survival are not included as it correlates to shoot length and mass respectively.

Table 1. Seedling growth and mycorrhizae infection as affected by soil type, inoculation and fungicide. 1/

		Shoot length (cm)	Total fresh mass (g)	% Infection	No. infected short
Soil	Sand	14,25***	2,80***	32,21***	91,33***
	Clay	5,48	0,73	6,48	5,54
Mycorrhizae	Control	7,16b	1,36b	7,44b	14,51b
	Pt.	8,77ab	1,61ab	10,45b	27,20b
	Rhiz.	10,97a	1,93a	28,24a	69,68a
Inoculum	Spores	9,26	1,28	17,50	53,71
	Veg.	10,48	2,26*	21,19	43,16
Fungicide	Control	11,30*	2,21*	23,98**	69,30***
	Kaptan	8,43	1,33	14,71	27,58

1/ Those means within a column not sharing a common letter differ significantly (P=0,05) by Scheffe's test. For main factors, means with *, **, *** are significantly bigger at P=0,05, 0,01, 0,001 respectively (F test).

There were no significant interactions for mass. For the other parameters, soil x mycorrhizae x inoculum interaction was highly significant: in sand Pt vegetative inoculum was better than spores but Rv spores were better than vegetative inoculum; in clay the inverse applied. For % infection and number of infected short roots, soil x mycorrhizae interaction was more marked in sand than in clay. For number of infected short roots, soil x fungicide interaction was significant: Kaptan had a negative effect which was more marked in sand than in clay.

The mean treatment values for all parameters were highly significantly better than the controls. There was a significant positive correlation between the number of infected short roots and shoot length.

Fungus mantles of the Rv inoculated seedlings were generally slightly thicker than those of the Pt inoculated and control treatments. Most of the Hartig-net hyphae penetrated to the endodermis.

A small portion of the ectomycorrhizae on the Pt inoculated seedlings appeared to be actually Pt. However, no Pt cultures were reisolated. Therefore, there is as yet no conclusive evidence that the Pt isolate used in this study is symbiotic with P.radiata. Rv was only reisolated from seedlings which were inoculated with Rv. A few unidentified cultures with clamp connections were isolated from control as well as Pt and Rv inoculated seedlings, i.e. the precautions taken to keep foreign ectomycorrhizal spores out of the potted soil, were not entirely effective.

In conclusion, the major factor affecting seedling growth and ectomycorrhizal infection was soil type, i.e. sand being better than clay; Rv inoculation significantly stimulated seedling growth but Pt inoculation did not; the application of Kaptan inhibited ectomycorrhizal infection and seedling growth.

EFFECT OF NUTRITION ON GERMINATION AND GROWTH OF VESICULAR-ARBUSCULAR MYCORRHIZAL (VAM) FUNGI

By

J.O. Siqueira and D.H. Hubbell

Keywords-- *Glomus mosseae*, *Gigaspora margarita* axenic culture, spore germination.

Introduction

VAM fungi are obligate biotrophs and have not been grown in the absence of living roots. This is the most challenging and critical limitation for intensive studies of the physiology, taxonomy, host relationships and practical utilization of these fungi. This paper reports the effects of several organic substrates on germination and germ tube (GT) growth of VAM fungal spores *in vitro*.

Material and Methods

Surface disinfected spores were transferred to 1% agar media containing 20 mg/l CaH_2PO_4 and 0.01 mg/l thiamine-HCl and the organic substrates as in Table 1. The effect of glucose was examined by applying 15 ml/plate of aqueous solution containing the desired concentration to sterile sand plates where spores were placed to germinate between two membrane filters. Soil extracts and root exudates were obtained as described by Siqueira (1983). They were subjected to molecular separation using a Bio-Rad P-10 gel column (1500-20000 K). The individual fractions were analysed for carbohydrate and protein and assayed for their effects on spores. Spore germination and GT growth were assessed according to Siqueira et al. (1982).

Results and Discussion

Table 1 shows some of the organic substrates with consistent effect on spore germination and GT growth. Many other substrates examined showed unconsistent results. In general, organic substrates were inhibitory to either germination or GT growth. In sand plates 0.6 g/l of D-glucose improved germination while concentrations higher than 0.8 g/l decreased (Fig.1). The GT growth process in less sensitive to glucose concentrations than is germination.

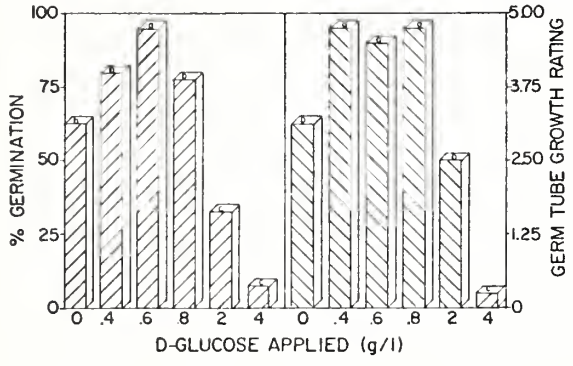

Figure 1. Effect of glucose on germination and GT growth of *Glomus mosseae* spores.

Soil extracts showed beneficial effects on GT growth of VAM fungi *in vitro* (Fig. 2).

Table 1. Effect of organic substrates on *Gigaspora margarita* spore germination and growth.

Substrate	Conc.	Germination	GT growth
	g/l	% of control	[1/]
Sucrose	1.0	99	86
Sucrose	4.0	86	125
Sucrose	16.0	58	40
Fructose	12.0	49	72
L-Arabinose	1.2	45	81
Aspartic acid.	2.0	39	54
Succinic acid	2.0	18	0
Tartaric acid	5.0	25	30
D-Gal. acid	0.5	98	33
D-Gal. acid	1.0	141	65
D-Gal. acid	4.0	27	30
Na-Acetate	0.3	118	95
Na-Acetate	0.6	68	71
Na-Acetate	1.2	0	-
Glycerol	4.0	50	0
Ca-Phytate	1.0	77	64
Casein hydrolysate	1.0	75	95
Peptone	1.0	100	55
1% soil extract agar		93	139
1% root exudate agar		64	103

1/ Control with 20 mg CaH_2PO_4 + 0.01 mg thiamine-HCl per liter = 100% for germination and GT growth.

This effect is associated with the protein content of the fraction. It is suggested that protein is the enhancing factor and it may be responsible for the limited saprophytic ability of these fungi in soil.

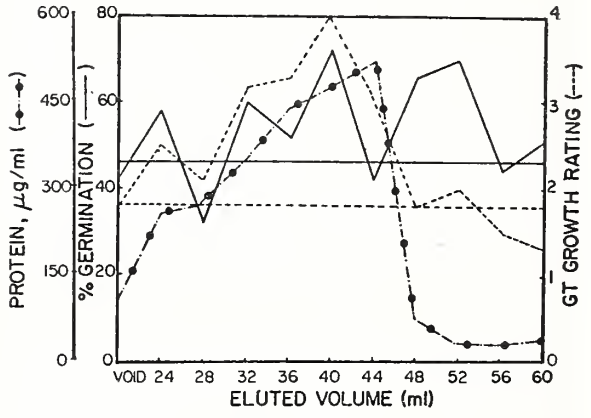

Figure 2. Protein content and activity of the soil fractions. The horizontal lines represent control treatments.

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RESPONSES OF ASPARAGUS SEEDLINGS TO THREE GLOMUS ENDOMYCORRHIZAL FUNGI

By

D.C.N.Chang

Keywords--UC 309, VAMF, mycorrhizal dependency, fertilizers

Introduction

Asparagus(Asparagus officinalis L.) is a very important profitable crop in Taiwan. Chen(1981) reported that Glomus mosseae vesicular-arbuscular mycorrhizal fungi (VAMF) could stimulate the growth of cv. UC 309 asparagus seedlings. In order to make VAMF practical in cultivating asparagus seedlings,an experiment was conducted to make certain:(1) the growth effects of 3 Glomus VAMF and the mycorrhizal dependency, and (2) fertilizer effects on the growth of mycorrhizal(M) and non-mycorrhizal(NM) asparagus seedlings.

Materials and Methods

The inocula consisted of soil and vermiculite(1:1),roots and spores from a pot cultured corn(Zea mays) which had grown for 3 months after being infected with pure line of Glomus etunicatum Becker & Gerd. or G. fasciculatum(Thaxter sensu Gerd.)Gerd. & Trappe or G. mosseae(Nic. & Gerd.)Gerd. & Trappe. The experiment was carried out in a phytotron kept at 30/25°C(day/night). Tests included:(1) inoculum: only one of the 3 Glomus spp. was inoculated for each treatment. Mycorrhizal dependency was expressed as the percentage of M plant dry mass over NM plant dry mass.(2) 5 fertilizer levels(in ppm): including full NPK(160 :40:120), half NPK(80:20:60),NK(160:120), N(160) and control total 5 fertilizer levels for both M and NM plants. There were 3 plants for each treatment, and the experiment was repeated for 4 times in 2 years. Fertilizers were applied twice per month. For inoculum test, only 2 fertilizer levels of full and half NPK were applied. A mixture of sand,vermiculite and soil(1:1:1) was used as growth media. The P content of soil was about 10 ppm. Five-month-old seedlings were harvested for analysis.

Results and Discussion

Table 1 showed that the growth of asparagus seedlings was significantly enhanced by all the 3 Glomus VAMF, and showed very high mycorrhizal dependencies. Plenchette et. al.(1982) reported that the growth of Mary Washington asparagus seedlings was significantly stimulated by Glomus epigaeus and Glomus monosporus. Thus it was concluded that asparagus was highly dependent on Glomus VAMF, and the inoculation with Glomus spp. would be beneficial to the cultivation of asparagus seedlings, especially in sterilized soil.

Table 1. Growth and mycorrhizal dependency of asparagus seedlings inoculated with 3 Glomus spp. fungi. 1/

Glomus spp.	Dry mass(g)of shoot	root	Mycorrhizal dependency(%)
etunicatum	4.92^a	6.16^a	1119.19^a
fasciculatum	4.37^a	6.21^a	1068.69^a
mosseae	4.95^a	4.90^b	994.95^a
Control	0.58^b	0.41^b	100.00^b

1/Those means within a column not sharing a common letter differ significantly(p= 0.05) by Duncan's multiple range test.

Comparatively, G. etunicatum and G. fasciculatum were better inocula for increasing the dry masses of shoot and root. Usually higher dry mass in both shoot and root resulted in a higher mycorrhizal dependency.

Fertilizer test results were shown in Table 2.

Table 2. Fertilizer effect on the growth of mycorrhizal and non-mycorrhizal asparagus seedlings.

Fertilizer levels(ppm)	Dry mass(g)of shoot	root	Number of stem	root
Full NPK (160:40:120)	4.25^a	8.61^a	10.44^a	35.22^a
Half NPK (80: 20: 60)	2.28^c	6.77^b	7.94^b	26.67^b
NK (160:120)	3.62^b	7.51^{ab}	8.27^b	29.72^b
N (160)	3.34^b	6.63^b	7.68^b	27.78^b
Control	0.44^d	1.68^c	4.50^c	9.67^c

Table 2 indicated that applying any level of fertilizer would significantly enhance shoot and root dry masses and the number of stem and root. Full NPK application resulted in the best growth for both M and NM plants. Both NK and N applications showed better growth than half NPK's treatment. Therefore, it was concluded that at least N fertilizer should be applied for cultivating asparagus seedlings for both M and N plants. Field tests should be conducted for this type of study.

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OCCURRENCE OF VAM WITHIN NODULES OF COMMON BEAN

by
R. M. N. Kucey, S. C. Peron, E. P. Portugal and S. M. T. Saito

Keywords: VAM colonization, Phaseolus vulgaris, latosol

Introduction

VAM fungi have been reported to occur in a wide variety of habitats. They have not yet, however, been reported to colonize N_2 fixing nodules of legumes, although the roots themselves are often heavily colonized with VAM. This report describes the occurrence of an unidentified VAM fungus which colonizes nodules of the common bean.

Materials and Methods

A Brazilian dark red Latosollic soil was mixed 1:1 with coarse silica sand. To three kilograms of soil in each pot was added N (20 µg/g), P (37.5 µg/g), K (47.2 µg/g), and S (7.5 µg/g). Three seeds of Phaseolus vulgaris var. Carioca, inoculated with Rhizobium phaseolus, were planted in each pot. VAM occurred indigenously in the soil. Plants from four pots were harvested at 15, 36, 58, and 79 days. Shoots were dried and weighed. The root systems were washed free of soil and subsampled for VAM colonization analysis. Nodules were picked from the roots. Root subsamples and nodules were cleaned, stained, and examined microscopically for VAM colonization.

Results and Discussion

An unidentified VAM, indigenous to the Brazilian soil, was found colonizing the nodules of Phaseolus vulgaris. Colonized nodules were found in root systems aged 36 to 79 days. The levels of colonization appeared to be roughly equivalent to the level of VAM colonization on the roots (Fig. 1). The VAM structures appeared similar in both roots and nodules. Hyphae were found penetrating the nodule directly (Fig. 2) and entering the nodule from the subtending root. Hyphae were found ramifying through the nodule and extending out into the soil (Figs. 3 and 4). Structures that were vesicles or small spores (100 µm) were found within the nodule. No arbuscules were found within the nodules themselves, however, the subtending root held many arbuscules, particularly around the point of attachment of the nodule.

VAM spores isolated from the soil included one species of Gigaspora, two species of Glomus, a "puzzle" type tentatively assigned to Acaulospora and an unidentified spore with a septate support hypha. No mature spores were found attached to colonized nodules.

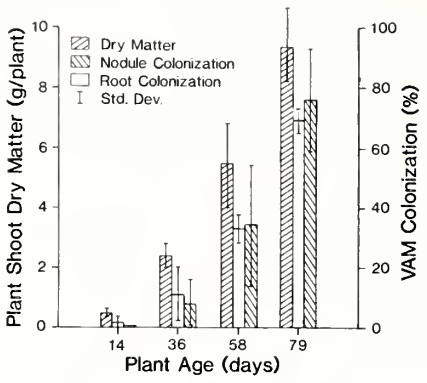

Figure 1. Plant dry matter production and levels of VAM colonization in roots and nodules.

Figure 2. Entry point of VAM into nodules (arrow)

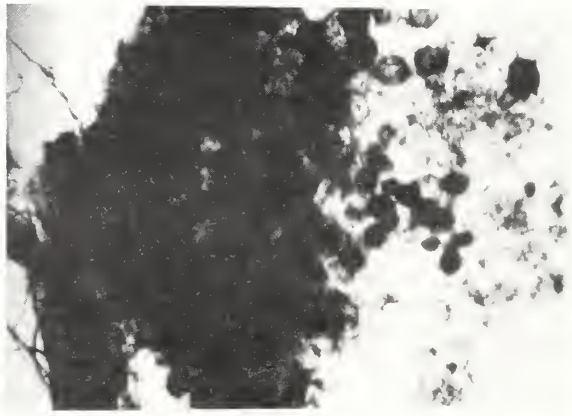

Figure 3. VAM colonized nodule.

Figure 4. VAM colonized nodule.

MYCORRHIZA AND PLANT WATER RELATIONS IN *ARACHIS HYPOGAEA*

By

K. R. Krishna and D. J. Bagyaraj

Keywords—Diffusive resistance, Drought, peanut, Proline

Introduction

The growth enhancement due to vesicular-arbuscular mycorrhiza (VAM) has been generally attributed to increased nutrient (especially phosphorus) uptake. Information on the effects of mycorrhizal infection on other physiological processes that influence growth, such as water relations is limited. The few studies conducted so far show that mycorrhizal inoculation reduces plants resistance to water transport (Safir, et. al.,1972) and that the mycorrhizal plants tolerate water stress better. Peanut is an important oil seed crop grown extensively in semi-arid tropics under rainfed conditions or sometimes with protective irrigation.

Methods and Materials

Peanut (*Arachis hypogaea*, cv DH 3-30) was grown in 15 cm pots filled with 3 kg of steam sterilised soil with and without mycorrhizal inoculum. The soil used was a red sandy loam with pH 5.5, deficient in P (3 mg available P per kg soil extractable with NH_4F+HCl). Mycorrhizal inoculum contained chlamydospores and root bits of *Panicum maximum* Jacq. which was infected with *Glomus fasciculatus* (Thaxt.) Gerd. and Trappe. The inoculum was placed 2 cm below the soil surface before sowing to produce mycorrhizal plants. Plants without mycorrhizal inoculum served as non-mycorrhizal controls. Establishment of mycorrhizal association was confirmed by staining the roots with trypan blue.

Pots were watered daily, to a constant weight, from sowing until 50th day and then allowed to dry. The drying phase extended for 54 hours when the plants showed signs of wilting. After 54 hours of drying phase, stressed mycorrhizal and non-mycorrhizal plants were rewatered. Soil moisture, relative water content, proline content and diffusive resistance of the leaves were determined 6, 30 and 54 hours after induction of the drying phase and also 24 hours after rewatering. Soil moisture in the root zone was expressed as per cent moisture present on oven dry basis. Relative water content, which is the water content (on a percentage basis) relative to the water content of the same tissue at full turgor was determined in the third fully opened leaf. Second fully opened leaf from apex was utilised for proline determination and diffusive resistanee measurement. Leaf proline content, which increases faster under stress, was estimated by the method of Bates (1973). Diffusive resistance, which is a measure of the relative stomatal was measured using a DL 60 LICOR diffusion porometer (Turner and Parlange, 1975).

Results and Discussion

Relative water content of the leaves of mycorrhizal plants were slightly higher than that of non-mycorrhizal plants during the drying phase (Table 1). Upon rewatering, the mycorrhizal plants regained leaf turgidity rapidly compared to non-mycorrhizal plants. The concentration of proline which increases faster under stress, was lower in mycorrhizal plants compared to non-mycorrhizal plants. Rewatering decreased proline content more rapidly in mycorrhizal plants. Levy and Krikun (1980) observed a slight reduction in the proline content of mycorrhizal rough lemon leaves compared to non-mycorrhizal plants only in the initial stages of water stress, which disappeared laster. Leaves from mycorrhizal plants had lower diffusive resistance indicating that their stomata were more open than those of non-mycorrhizal plants. This was true both during the drying phase as well as upon rewatering. Upon rewatering stomatal resistance dropped faster in mycorrhizal plants compared to non-mycorrhizal plants. Lower leaf resistances to water vapour diffusion in mycorrhizal rangeland grass have been observed (Allen et. al. 1981). It has been suggested that the reduced resistance could have resulted from enhanced water status of mycorrhizal plants. Rapid recovery of mycorrhizal red clover from water stress upon rewatering has been attributed to lower leaf water potential and higher root conductivity (Hardie and Leyton, 1981). Further, leaves from mycorrhizal rangeland grass have been shown to contain elevated cytokinin levels which has a role in regulating stomatal opening.

The present study clearly brings out that mycorrhizal inoculation influences the water relations of peanut. The observation that 24 hours after rewatering mycorrhizal plants recorded higher relative water content, lower amounts of proline and lowered stomatal resistance suggest that recovery from water stress is more rapid in mycorrhizal plants. Mycorrhizal inoculation could be of benefit to peanut which is grown extensively in semi-arid regions without irrigation depending on natural rainfall or with only protective irrigation to save the crop from drying.

Table 1. VA mycorrhiza and water relation parameters of peanut

		Inoc.	Hours after Stress 6	Stress 30	Stress 54	Rewater 24	SE
Soil moisture(%)		M	9.9	4.8	4.0	11.9	±0.52
		NM	10.0	4.4	3.9	11.2	
RWC(%)		M	87.0	57.7	25.1	85.0	±6.2
		NM	84.4	44.2	21.0	82.0	
Proline µg/g d.wt.		M	333	2050	6564	919	±123.5
		NM	564	2159	6722	1104	
Diffusive resistance (sec/cm^{-1})	LS	M	1.7	27.0	ND	5.6	±6.0
		NM	7.8	51.0	ND	14.5	
	US	M	2.7	51.0	ND	2.0	±4.8
		NM	6.2	63.0	ND	19.3	

Plant age: 52 days; M=inoculated with mycorrhiza; NM=Uninoculated;ND=Not determined;RWC=Relative water content; LS=Lower surface; US=Upper surface

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PHOSPHATASES IN THE RHIZOSPHERES OF MYCORRHIZAL AND NON-MYCORRHIZAL PEANUT

By

K.R. Krishna and D.J. Bagyaraj

Keywords--Arachis hypogaea, endomycorrhizas, Rhizosphere, Soil phosphatase

Introduction

The role of mycorrhizas in phosphorus nutrition of the host plant is now well established (Hayman, 1980). Phosphatases produced by plant roots and microorganisms (including mycorrhizal fungi) in soil have been implicated in the release of phosphate from the organic esters. Macdonald and Lewis (1978) recorded acid phosphatases in the external mycelium of vesicular arbuscular (VA) mycorrhiza. Mycorrhiza specific alkaline phosphatases (MSAP) in mycorrhizal symbiotic efficiency (Gianinazzi-Pearson and Gianinazzi,1978). There are no reports on the soil phosphatase in rhizosphere of mycorrhizal and non-mycorrhizal plants.

Methods and Materials

Red sandy loam soil of low fertility (organic C, 0.35%; total N, 0.04% NH_4F-HCl extractable P, 5ug. g^{-1}) and pH 5.6 was used. Phosphatases were studied both in sterilized and unsterilized soils. Soil was sterilized by autoclaving. Groundnut (Arachis hypogaea L.)was grown in plastic pots of Glomus fasciculatum was used as the mycorrhizal inoculum. The inoculum (300 spores surface sterilized with 1% $NaHClO_3$ for 5 min/seedling hole) was placed 2 cm below the surface of the soil before sowing. Plants without inoculation served as controls. Non-rhizosphere soil was obtained by maintaining pots without plants throughout the period of the experiment. There were 24 replicate pots for each treatment.

Six pots from each treatment were harvested 15,30,45 and 60 days after sowing. Mycorrhizal root infection was checked at each sampling date. Soil acid phosphatase was estimated according to Tabatabai and Bremner (1969). Enzyme activity was expressed as ug PNP activity was determined in a similar manner.

Results and Discussion

In sterilized soil, acid phosphatase activity in the rhizospheres of mycorrhizal and non-mycorrhizal plants was significantly higher than in non-rhizosphere soil, at all sampling dates (Table 1). The enzyme activity in the sterilized non-rhizosphere soil indicates non-microbial phosphatases 'force' in soil. No significant differences were noted between mycorrhizal and non-mycorrhizal plants. Similar trends were also observed in alkaline phosphatase activity. In unsterile soil, differences in alkaline phosphatase activity among the three treatments were not significant (Table 2). With acid phosphatases, however, the activity was at two harvests in the rhizosphere of mycorrhizal plants followed by non-mycorrhizal rhizosphere and then the non-rhizosphere soil. These results confirmed the earlier findings of Ridge and Rovira (1971), that the presence of active plant roots enhances the activity of soil acid and alkaline phosphatases.

The acid phosphatase activity in the rhizospheres of mycorrhizal plants in unsterile soil was significantly higher than in those of the non-mycorrhizal plants only in the early stages of plant growth (days 15 and 30). We have found previously that mycorrhiza stimulated the microbial activity and longevity of some specific organisms including P solubilizers in the rhizosphere (Bagyaraj and Menge, 1979; Raj et al., 1981). Thus along with the various mechanisms postulated and tested so far to account for the increased P absorption by mycorrhizal plants the possibility of stimulated microflora in the mycorrhizosphere may also considered. It is possible that MSAP occurring in mycorrhizal plant roots have a role to play in P transfer at the hypha-cytoplasm interface. Further research efforts are needed on the sigmatic mechanisms involved in P transfer to plants at soil-root interface, and the possible role played by the enzyme in mycorrhizosphere.

Table 1. Soil acid and alkaline phosphatase activity in the rhizosphere of groundnut grown in sterilized soil

Plant age in days	% Mycorrhizal infection		Soil acid phosphatases (µg PNP released, g^{-1} soil h^{-1})			Soil alkaline phosphatases		
	M	NM	M	NM	NR	M	NM	R
15	0	0	304	267	213	128	124	110
30	58	18	299	235	218	126	127	105
45	72	38	231	218	210	122	121	103
60	82	60	225	223	195	114	110	108
LSD at P=0.05			30.72			NS		

M - Mycorrhizal rhizosphere; NM - Non-mycorrhizal rhizosphere; NR - Non-rhizosphere

Table 2. Soil acid and alkaline phosphatase activity in the rhizosphere of groundnut grown in unsterilized soil

Plant age in days	% mycorrhizal infection		Soil acid phosphatases (µg PNP released g^{-1} soil h^{-1})			Soil alkaline phosphatases		
	M	NM	M	NM	NR	M	NM	NR
15	0	0	198	202	153	112	113	70
30	56	0	178	173	148	107	106	70
45	68	0	164	161	123	94	93	60
60	77	0	166	165	128	96	88	80
LSD at P=0.05			24.21			28.57		

M - Mycorrhizal rhizosphere; NM - Non mycorrhizal rhizosphere; NR - Non rhizosphere

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DEVELOPMENTAL EFFECTS ON MICRONUTRIENT DISTRIBUTION IN MYCORRHIZAL AND P-FERTILIZED SOYBEANS

R. S. Pacovsky, G. Fuller, and E. A. Paul

Keywords- *Glycine max*, *Glomus mosseae*, *Glomus fasciculatum*, iron, manganese, copper, zinc

Introduction

Colonization of soybeans by vesicular-arbuscular mycorrhizal (VAM) fungi often results in the enhanced uptake of relatively immobile nutrients. Evaluation of nutritional differences is complicated by two factors: 1) non-VAM control plants are often not given compensating fertilizer and 2) the timing of the evaluation may accentuate transitory differences. A one-time harvest with inadequate controls could lead to incorrect inferences about the overall nutrient uptake and allocation processes in mycorrhizal symbioses. Although the major VAM effects are attributable to P uptake, many physiological and biochemical reactions involve micronutrients (Fe,Mn,Zn,Cu), and in nodulated legumes these cofactors are essential for nodule growth and N_2 fixation. Therefore,we determined micronutrient uptake in VAM plants as compared to P-fertilized non-VAM soybeans throughout plant ontogeny.

Material and Methods

Soybean (*Glycine max* L. Merr. cv Amsoy 71) plants were grown in a greenhouse in an acidic, low available P soil The plants were inoculated with a VAM fungus (*G. mosseae or G. fasciculatum*) or received a nutrient solution containing one of three P concentrations (0.0, 0.2 or 1.0 mM P). Five replicates from each treatment were selected at random and harvested after 3, 6, 9, 12 and 21 weeks of growth. Plant digests were analyzed for Fe, Mn, Zn and Cu by atomic absorption spectrophotometry and for P by colorimetry. Roots were stained and VAM-fungal colonization assessed.

Results and Discussion

There was significant growth enhancement in terms of plant dry weight and P content for VAM plants compared to the control (0.0 mM P). Dry weights for VAM plants were greater than those for 0.2 mM P-fertilized plants prior to week 12, similar at week 12, and significantly less afterwards. Differences in P content between the VAM and 0.2 mM P fertilized plants were significant only at week 3 and 21. Non-VAM plants that compare to VAM-inoculated hosts in terms of phytomass and P nutrition are the most suitable controls under these conditions.

Micronutrient concentrations varied among soybean plant parts throughout development. The relative uptake and allocation of micronutrients was significantly modified in VAM plants compared to the 0.2 mM P treatment (Table 1). The Fe and Mn concentrations in VAM plants were lower by 40 to 50% during host-plant ontogeny. Acid soils often release phytotoxic levels of Mn, but VAM-fungal colonization moderated the uptake to less than critical levels. Copper and Zn were consistantly higher in VAM plants throughout development, though differences in root Zn content were not significant after week 6. The differences in micronutrient concentrations for fully-developed soybean pods were similar to differences in leaves and roots. This indicates that the effect due to VAM fungi in altering mineral allocation occurs not only in vegetative structures, but in mature pods as well.

Table 1 Differences in micronutrient content between VAM and 0.2 mM P-fertilized plants. 1/

Plant part	Week 3	6	9	12	21
	- - - - - - % difference - - - - - -				
			Iron		
Leaf	-4	-28*	-34*	-21*	+5
Root	-3	-28*	-48*	-46*	+22
Pod	ND	ND	-27*	-12	-18
			Manganese		
Leaf	-1	-8	-43*	-35*	-24*
Root	-17*	-15*	-32*	-33*	-12
Pod	ND	ND	-49*	-44*	-37*
			Zinc		
Leaf	+37*	+74*	+65*	+21*	+34*
Root	+48*	+27*	+9	+18	+16
Pod	ND	ND	+2	+42*	+36*
			Copper		
Leaf	+28	+56*	+88*	+34*	+46*
Root	+62*	+25*	+51*	+19*	+40*
Pod	ND	ND	+14	+51*	+49*

1/ Percent differences were calculated as: ([VAM - P]/P) x 100. ND = no data.
VAM values are means for both fungal associations.
*indicates significant difference at $p < 0.05$.

In general, differences in nutrient content increased until flowering (week 9), then declined. Micronutrients were taken up rapidly during vegetative growth then declined during soybean pod development. Plant growth requires an adequate supply of minor elements early in development when VAM fungi are most active. This emphasizes the role of mycorrhizae in increasing the efficiency of mineral assimilation.

Endomycorrhizal biomass and percent infection were greater in plants inoculated with *G. fasciculatum*. These plants had increased dry weight, P content and micronutrient differences relative to plants colonized with *G. mosseae*.

Conclusions

Usually VAM-colonization is advantageous to plant growth by providing limiting nutrients, such as P, Zn, and Cu. A more novel effect is the decreased Fe and Mn uptake by VAM plants. The mechanism by which VAM controls Mn uptake is not known. Low concentrations of available Fe and Mn may occur in VAM hyphae to avoid the precipitation of insoluble phosphates can occur. Of interest is the increased Zn and Cu concentration in VAM plants. It is uncertain if this increase is the result of higher requirements for these elements by VAM-colonized hosts, or if accumulation is incidental to the greater uptake capability of the fungus.

C-FLOW AND N_2 FIXATION IN *SORGHUM*- *AZOSPIRILLUM*-*GLOMUS* ASSOCIATIONS.

By

D. Harris, R.S. Pacovsky and E.A. Paul

Keywords--Photosynthesis, 15Nitrogen,14Carbon, isotope dilution.

Introduction

The presence of VAM in the legume - *Rhizobium* symbiosis increases nodulation and N_2 fixation. These triple symbionts have increased beneath ground allocation of C and net rates of photosynthesis which may compensate for the C requirements of the microbial symbionts. Colonization of C4 grass roots by N_2-fixing bacteria such as *Azospirillum* sometimes improves plant growth and N uptake.

This study examines the *Sorghum*-*Azospirillum*-*Glomus* association for:-
1. 15N uptake and fixation by isotope dilution.
2. $15N_2$ fixation.
3. $14C$ uptake and allocation by $14CO_2$ pulse-chase labelling.

Methods and Materials

Sorghum bicolor was grown in pots in a sterilized P-fixing soil. The plants were inoculated with *A. brasiliense* or *Glomus fasciculatum*, with both, or with soil washings sieved free of mycorrhizal propagules. Plants without symbionts were given N and P fertilizer to compensate for the effects of the symbionts. For isotope dilution measurements, $15(NH_4)15NO_3$ was added in nutrient solutions to give total additions of either 94.8 or 0.9 mgN/pot. At 10 weeks, plants were pulse labelled with $14CO_2$ (200 uCi $g^{-1}C$) at 350 ± 20 ppm for 16h. The label was 'chased' by a further 4 days incubation in $12CO_2$. CO_2 respired below ground was collected. For the final 40h of the incubation, $15N_2$ was injected into the recirculating below ground atmosphere, of plants not given 15N fertilizer.

Results and Discussion

Isotope dilution measurements in plants with *Azospirillum* alone (Table 1) showed fixation or enhanced soil N uptake of 18 mgN/plant in a 10 week growth period. This represented 20% of total N in plants given trace amounts of 15N. Infection by *G. fasciculatum* alone resulted in apparent N_2 fixation of 5.4 mg/plant, when compared to the non-mycorrhizal control, probably due to greater uptake of soil N by the mycorrhizae rather than fixation. Therefore, mycorrhizal plants were used as non-fixing controls to calculate N_2 fixation in plants with both endophytes. The presence of mycorrhiza resulted in a 60% decrease in N_2 fixation by *Azospirillum*. In the short-term assay of $15N_2$ fixation, plants with *Azospirillum* alone fixed 5 times more $15N_2$ than plants which were also mycorrhizal (65 and 12 ugN/plant/day). About 50% of the 15N was found in the plant shoots indicating rapid transfer and uptake by the plant.

Table 1. N_2 fixation in Sorghum measured by isotope dilution.

Symbionts	Fert.15N	Total N	N_2fixation
	----------	(mg/plant)	----------
None	95	186	0
Azo.	95	189	18
Azo.	0.9	88	18
Azo.+VAM	95	175	8 (13)#
VAM*	95	164	0 (5)#

* used as control for Azo+VAM plants
\# using non-VAM plants as control

The presence of VAM increased the allocation of C to the root system by 4-7% of the total C flux, the additional C was recovered as respired CO_2. In contrast to other mycorrhizal and legume symbioses, the increase in below ground respiration was accompanied by a decrease in $14CO_2$ uptake in the leaves (Table 2). Effects on leaf area and leaf N and P concentrations were small.

Table 2. Net C fixation in *Sorghum*-*Azospirillum*-*Glomus* associations.

Symbiont	None	Azo	VAM	Azo+VAM
Net fixation ($mgCg^{-1}h^{-1}$)	3.0	2.4	2.3	2.3
Specific leaf area (dm^2g^{-1})	197	201	207	200
Leaf N %	0.91	0.94	0.93	0.91
Leaf P %	0.13	0.15	0.17	0.14

Azospirillum increased intraradicle mycorrhizal infection (2.1 to 2.7% root dw). Mycorrhizal plants were less extensively colonised by *Azospirillum* than those without VAM which agrees with the lower N_2 fixation rates measured in tripartite symbiosis.

These results show complex interactions between symbionts in tripartite association. The stimulation of mycorrhizal development and accompanying decrease in colonization by *Azospirillum* combine to suppress N_2 fixation in mycorrhizal plants. These interactions may provide an explanation for some of the variability found in rates of associative N_2 fixation. The reduced rates of photosynthesis found in plants with mycorrhiza, *Azospirillum*, or both, suggest that the growth rates of these plants were declining in comparison to uninoculated, nutrient-compensated controls, at the time of measurement. In future experiments it may be necessary to vary rates of nutrient addition to more closely match the growth curves of symbiotic plants.

SUPPRESSION OF SPORE GERMINATION OF VA MYCORRHIZAL FUNGI IN NATURAL SOIL AND POT CULTURES

By I.C. Tommerup

Keywords--Acaulospora laevis, Glomus caledonium, Gigaspora calospora, spore survival, germination inhibitors

Introduction

Spores of VAM fungi can germinate in some natural soils but not in others, although the chemical and physical properties are conducive. This behaviour is indicative of fungistasis or some other form of germination suppression. Elucidation of any role inhibitors may have in regulating germination would contribute to an understanding of spore survival and life history strategies under natural vegetation, in agricultural crops, pot cultures or stored soils.

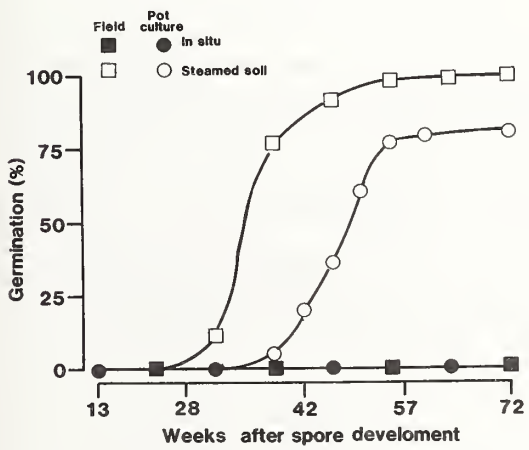
Methods

Spore germination in situ under crops, in pot cultures, or stored, moist field and pot culture soil was compared with spores when transferred to other agricultural or forest soils. Biological and chemical factors associated with suppression or inhibition of germination were investigated.

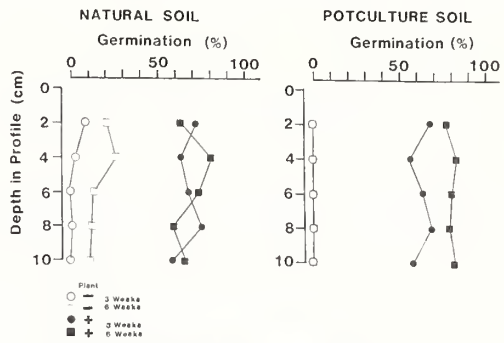
Results and Discussion

In situ germination of Glomus caledonium, Gigaspora calospora and Acaulospora laevis was suppressed in soils under crops, pastures, or in pot cultures. Spores did not germinate when stored in these soils. Spores separated from pasture soils did not germinate in wheat soils. Initially soil from under a forest was not suppressive but became so after one subterranean clover crop. In all soils suppression of germination was relieved by a crop of seedlings or by treatment with heat or methyl bromide.

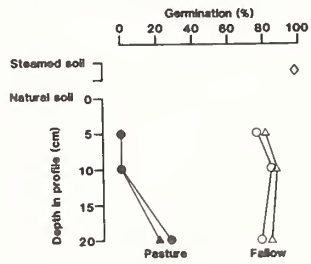
FAILURE OF SPORES OF ACAULOSPORA LAEVIS TO GERMINATE IN THE SOIL IN WHICH THEY FORMED.



INSITU GERMINATION OF QUIESCENT SPORES OF GLOMUS CALEDONIUM IN THE ABSENCE OR PRESENCE OF PLANTS.



GERMINATION OF QUIESCENT SPORES OF GIGASPORA CALOSPORA AT VARIOUS DEPTHS IN THE PROFILE UNDER A PASTURE OR IN A FALLOW STRIP IN THE PASTURE.



Small, water soluble, heat labile compounds which prevented germination were extracted from the suppressive crop or pot culture soils but not from forest soil. Water soluble inhibitors of germination which were closely associated with the spores also appeared to regulate germination. Microorganisms in association with old roots may contribute to suppression or alleviate it with young roots. Bacteria from the spore surface promoted germination in extracts of suppressive soils. Regardless of the mechanisms, prevention of in situ germination during the growing season in which spores formed and when stored would contribute to the maintenance of the population. This is of practical significance to methods for the large-scale production of inoculum and to persistence of the fungi under diverse ecological conditions.

Table 1. Spore germination of G. caledonium on soil extracts with or without chloramphenicol.

Soil	Extracted at °C	Dialysed	Germination (% at 2 weeks) Chloramphenicol (100µg/ml)	Nil
Pasture	20	-/+	0/0	52/64
	80	-/+	83/78	86/82
Forest	20	-/+	62/71	90/87
	80	-/+	69/76	84/79
Nil	Nil	-/+	73/69	87/74

Table 2. Elimination of germination inhibitor of G. caledonium by washing.

Spores from fractionated soil	Germination (%) in steamed soil 2 weeks	4 weeks
washed x1	0	18
washed x4	88	89

STRATEGIES AND TACTICS FOR THE AXENIC CULTURE OF V-A MYCORRHIZAL FUNGI

P.G. Williams

Key Words: biotrophic fungi, rust fungi, physiology, nutrition, morphogenesis of fungi.

Introduction

Methods for the isolation of fungi from nature and their cultivation on artificial media have been available for a little over a century. Many fungi from a variety of habitats can now be grown in axenic culture. However, reports of the successful culture of fungi forming V-A mycorrhiza have proved to be apochryphal. The V-A mycorrhizal fungi are obligate biotrophs. It would therefore seem sensible in tackling this problem to look at how the culture of other obligate biotrophs was achieved; what technical obstacles were overcome that impeded previous attempts and what has been learned about the underlying processes that make this kind of fungus difficult to culture?

Fungi in the Uredinales (the rust fungi) are obligately biotrophic, parasitic symbionts whose axenic culture defied the efforts of several generations of mycologists. The culture of a small number of rust fungi was finally achieved about 25 years ago. The methods used are evidently crude because the majority of rust fungi are still difficult or impossible to culture (Maclean 1982). Nonetheless, several insights have been gained (Williams 1984) which may be helpful in obtaining the development of V-A mycorrhizal fungi in axenic culture.

Strategies and Consequent Tactics

Below is a list of five strategic considerations (S.I-V) which are based on hypothetical or actual attributes of V-A mycorrhizal fungi. With each strategic viewpoint is a brief outline of possible tactical manoeuvres (T.1,2,3, etc.).

S.I. Any "successful" method of culture is likely, in the first instance, to provide sub-optimal conditions for growth. Ability to grow under these conditions is a genetically determined, variable character; i.e. some strains will be easier to culture than others.
T.1. Base an axenic culture program on as many different genotypes (genera, species, strains of species) as is practicable.

S.II. Fungal growth rate in the host is slow (about 0.6 mm/d). Initially, saprotrophic growth is likely to be much slower.
T.1. Incubate cultures for long periods (months, not weeks). T.2. Abandon linear extension of hyphae for growth assessment; adopt a vitality estimate (optical, metabolic, etc.).

S.III. The outgrowth tube from a fungal spore is either a mycelial primordium (produces vegetative mycelia directly) or a promycelium (forms vegetative mycelia only after host penetration)(De Bary 1887). If the tubes formed by V.A. endophyte "spores" represent the latter case:
T.1. Determine signals needed to trigger formation of endophytic state in axenic culture. Note: Ability of spores to respond to signals is affected by conditions of spore production. T.2. Abandon spores as inoculum; use endophytic hyphae (in segments of infected roots from monoxenic cultures) as inoculum for axenic cultures.

S.IV. Axenic mycelia are likely to leak because of a membrane defect. This results in the loss to the external medium of metabolites which the fungi can synthesize but not retain within their cells in sufficient concentrations for growth (c.f. rust fungi, malarial amoebae, mammalian and plant cells in cell culture). An effective nutrient medium will contain an appropriate balance of substances (amino and organic acids, bases, vitamins, etc.) to compensate for the leakage. It will also provide compounds for which the mycorrhizal fungus is truly heterotrophic e.g. reduced N and/or S, thiamin, biotin etc.
T.1. Define and repair the membrane defect causing the leakage. T.2. Identify the leaking metabolites and formulate a compensating medium. T.3. Test many types and brands of natural products or synthetic mixtures of bio-chemicals. T.4. Experiment with the ratio of inoculum size/volume of medium. T.5. Investigate crossfeeding through co-culture with culturable fungi, bacteria.

S.V. Mycelial growth in culture may be subject to inhibition by substances present in media constituents, formed by autoclaving or generated by the mycelia themselves.
T.1. Supplement nutrient media with adsorbents (activiated charcoal, alumina, polyvinylpyrrolidone, serum albumin, etc.). T.2. Use a flowing nutrient arrangement.

Conclusions

Time has shown that the axenic culture of V-A mycorrhizal fungi is not a problem amenable to simple solutions. The successful technique will combine remedies to several physiological (and perhaps morphogenetic) processes that operate simultaneously to prevent growth of mycelia under the conditions prevailing in conventional fungal culture systems. The measures suggested here are not a blueprint for success but they might help. I would be grateful for comment and criticism.

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EVALUATION OF EXTRA- AND INTRARADICAL MYCELIUM DEVELOPMENT IN DIFFERENT VA ENDOPHYTES.

Schubert A. and Grippiolo R.

Keywords - endophyte efficiency, Glomus caledonium, Glomus clarum, Glomus fasciculatum

Introduction.

Selection of VA endophytes for their efficiency in increasing host growth can be made easier if parameters intrinsic to the fungus can be related to its usefulness to the plant. In this work the mycelium development of 3 endophytes was studied, to assess if the E/I index (extra-/intraradical mycelium rate), proposed by BETHELENFALVAY et al.(1982), or another related index can be used for this purpose.

Methods and materials.

Onion seedlings were inoculated with G. caledonium, G. clarum and G. fasciculatum respectively; inoculum was equalized on the basis of its inoculum potential (PORTER, 1979). Plants were flushed with nutrient solution containing 5 ppm P and were harvested at 15 days intervals. At each harvest root and shoot weight were measured; the quantity of mycelium was assessed in soil and root samples by glucosamine determination (RIDE and DRYSDALE, 1972). Standard errors were calculated from 5 replicates.

Results and discussion.

Inoculated plants grew more than controls; differences in shoot dry weight among treatments were not significant (tab. 1).
Mycelium could be detected in roots after 6 weeks (fig. 1); when hyphal growth was plotted against root weight, G. fasciculatum showed an initial fast development followed by a 'plateau' level of infection, whereas in the other species the mycelium grew steadily (fig. 1).
External mycelium development began earlier for G. fasciculatum (week 4); in all treatments extraradical fungal structures per unit root weight grew at a high rate until week 6, then mycelium amounts decreased or, in the case of G. clarum, increased at a lower rate (fig.2). The same was true when external mycelium/soil dry weight was considered.
As a consequence, starting from week 6 on, the amount of internal mycelium/plant increased steadily for all endophytes, whereas that of external mycelium/plant decreased, or increased slightly in the case of G. clarum, the E/I rate thus decreasing in all cases. These data suggest that an early arbuscular phase can support a large external mycelium growth, whereas later a decreasing fraction of arbuscular infection may account for the E/I rate decrease.
Among different parameters related respectively to plant growth and mycelium development, relative growth rate (RGR) and E/I showed good correlation in the case of G. caledonium and G. fasciculatum, but not for G. clarum. In this case factors here not controlled (fraction of internal mycelium in arbuscular phase and amount of external hyphae actively taking up P) may be playing a larger role. Parameters taking into account these factors could better express an endophyte's ability to enhance host growth.

Table 1. Shoot dry weight (mg)

week	C	GF	GC	GCL
2	6	6	7	5
4	6	10.2	9	8
6	11.5	17.7	18.2	20
8	18	28	25.4	24.2
10	19.5	34.4	27.8	38.2

C = control GCL = G. clarum
GC = G. caledonium GF = G. fasciculatum

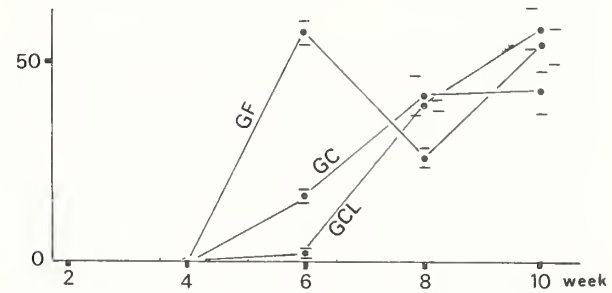

Fig. 1 Intraradical mycelium (µg glucosamine/g root fresh weight).
For abbreviations see tab. 1.

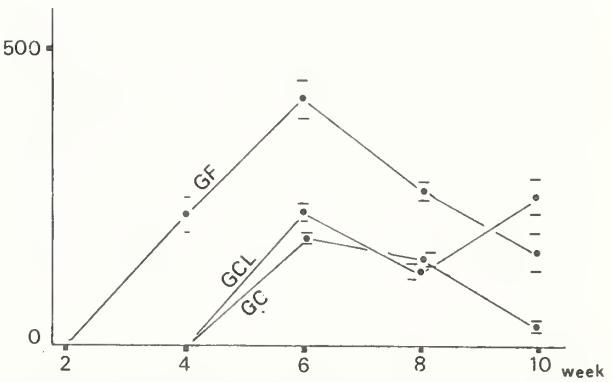

Fig. 2. Extraradical mycelium (µg glucosamine/g root fresh weight).
For abbreviations see tab. 1.

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STUDIES ON VA FUNGI ISOLATED FROM ENZYMICALLY DIGESTED MYCORRHIZAL ROOTS

By

V. Gianinazzi-Pearson, P. Bonfante-Fasolo, J. Dexheimer and S. Gianinazzi

Keywords--Histochemistry, ultrastructure, VA mycelium, Glomus, Gigaspora

Introduction

A problem for research on the physiology of VA fungi is the obtention of sufficient amounts of active mycelium without the spore or host plant. Capaccio and Callow's (1982) method of isolating mycelium from enzymically digested mycorrhizal roots could offer a solution. However, it is necessary to be sure of the physiological state of such mycelium before using it for detailed physiological studies.

Methods and Materials

Enzymic digestion of 0.5 to 1cm pieces of mycorrhizal root by incubation in 0.1M MES buffer, pH 6.0, containing cellulase (30mg . ml^{-1}), pectinase (15 units . ml^{-1}) and sorbitol (100mg . ml^{-1}) during 16h at 23°C successfully digested the walls of the parenchymatous cortical cells of Ornithogalum umbellatum and onion roots (Liliaceae) but not those of clover, raspberry, Ginko biloba or grapevine roots. After enzyme digestion, VA fungal mycelium was left free in the space remaining between the exodermis, hypodermis and central cylinder of roots and hyphal masses could be easily isolated . VA fungi studied were Glomus mosseae, G. fasciculatus (2 isolates), G. monosporus, Glomus E_3 and Gigaspora margarita.

Results and Discussion

Histochemical analyses were made to test the physiological integrity of the isolated mycelium after washing well in 0.1M MES / sorbitol solution (Table 1).

Extraction and electrophoretic separation of alkaline phosphatase activity in the isolated mycelium showed that it was equivalent to the mycorrhiza specific phosphatase (MSP) activity in infected root extracts (Gianinazzi-Pearson and Gianinazzi, 1976, 1978 ; Gianinazzi-Pearson et al. 1978).

Electron microscope studies of isolated mycelium revealed that :

. hyphae were not always free of host protoplasm which was sometimes entrapped between arbuscule branches ;

. the fungal cytoplasm was affected by the digestion process, mitochondria structure was altered and the plasmalemma fragmented into membranous vesicles. This could explain the poor results for succinate deshydrogenase activity in the mycelium ;

. the fungal vacuole, on the contrary, appeared to retain its integrity with the tonoplast intact ; alkaline phosphatase activity and polyphosphate-like granules were detected within the vacuoles, as in hyphae in mycorrhizal roots (Gianinazzi et al. 1979 ; Cox et al. 1980).

In conclusion, the structural disorganization of the fungal cytoplasm in mycelium isolated from digested roots greatly limits any interpretations that could be made on its physiology. This experimental approach could, however, be of interest for studies on certain physiological activities ; for example, alkaline phosphatase activity varied between arbuscules from the same mycorrhizal root which suggests that arbuscules of similar morphology can differ physiologically. The observations made on the fungal vacuoles suggest that such isolated VA mycelium may also provide suitable material for isolating these organelles for more detailed studies of their properties.

Table 1. Some histochemical characteristics of VA mycelium isolated from enzymically digested mycorrhizal roots

Test	Fungal structure			
	H	A	V	I
a)Total carbohydrates (Periodic acid-Schiff)	+	+	+	+
b)Neutral lipids (Sudan III)	+	−	+	+
c)Nuclei	+	+	+	+
d)Succinate deshydrogenase (Nitro blue tetrazolium)	±	±	±	±
e)Polyphosphate (Toluidine blue, pH 2.0)	+	+	±	+
f)Alkaline phosphatase (Azo dye coupling, pH 8.5)	+	+	±	−

+ present ; − absent ; ± weak or occasional staining.
H, hyphae ; A, arbuscules ; V, vesicles ; I, internal spores of G. fasciculatus.

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EFFECT OF POT-CULTURE AGE ON SPORE GERMINATION IN GLOMUS MOSSEAE

By

Kay Hardie

Keywords-- ectocarpic, sporocarpic
spore storage, contamination

Introduction

It was observed that, during trials on ectocarpic spore germination of Glomus mosseae (Nicol. & Gerd.) Gerdemann & Trappe, the onset of germination and the number of germinating spores was apparently influenced by the age of the pot-culture from which they were isolated. Experiments were conducted to investigate this phenomenon and to evaluate the effect of low temperature storage on spore germination.

Methods and Materials

Two isolates of Glomus mosseae, one from Rothamsted Experimental Station (Hertfordshire, England) and one from Wytham Field Station (Oxfordshire, England) were maintained in pot-culture on the roots of leek plants. The contents of pot-cultures of various ages were individually wet-sieved and decanted. Sporocarps were separated under a binocular microscope and ectocarpic spores were isolated by the sucrose flotation method(1). Table 1 summarises the various age, origins and treatments of spores prior to germination tests.

Table 1. Summary of Glomus mosseae spore treatments prior to germination experiments.

Treatment	Age of pot-culture (months)	Time at 7 - 8 °C (weeks)	Spore* type	Isolate
1	5	0	E	Rothamsted
2	5	5	E	Rothamsted
3	10	0	E	Rothamsted
4	10	5	E	Rothamsted
5	12	7	E	Rothamsted
6	12	7	S	Rothamsted
7	12	7	E	Wytham
8	12	7	S	Wytham

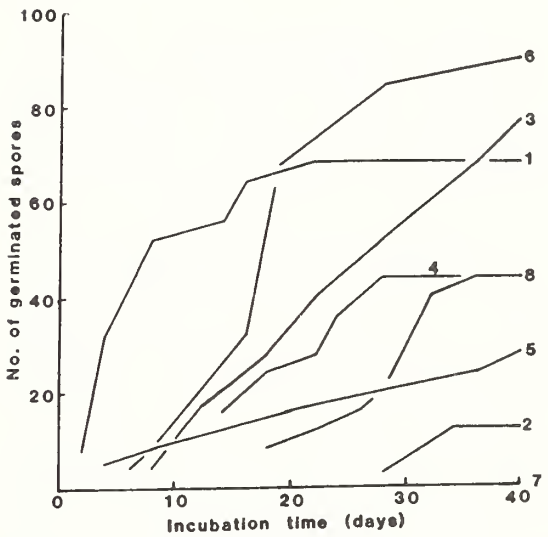
*E = Ectocarpic S = Sporocarpic

Surface sterilised spores were placed on water or nutrient agar, pH 6.9, in 'Replidish' compartments. From each treatment group 100 spores were transferred to water agar and 100 to nutrient agar. 'Replidishes' were incubated at 25°C in the dark.

Results and Discussion

Freshly isolated ectocarpic spores gave good germination results on water agar (Fig. 1), those from the youngest culture-pots germinating most rapidly. Storage of the spores reduced germination. Stored, ectocarpic spores from 12 month old culture-pots showed comparatively poor germination and were easily overshadowed by

Fig. 1. Effect of pot-culture age, spore storage and spore type on germination potential of G. mosseae. See Table 1 for designation of treatment nos.



similarly aged and treated sporocarpic spores. Sporocarpic spores from the Rothamsted isolate, stored at 7-8° C for 7 weeks, produced the highest number of germinated spores.

Incubation on a nutrient medium tended to inhibit germination in most spore treatments in agreement with other published data (2,3).

Fungal contamination was greatest in spores isolated from the youngest pot-cultures and decreased as pot-culture age increased. This reflects the higher susceptibility of young spores to invasion by soil microorganisms. As the population ages, spore walls become thicker and less susceptible to attack. Thus the absence of contamination in ectocarpic spores from the oldest pot-cultures was a reflection of the initial selection of undamaged, healthy spores.

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Autofluorescence of a vesicular-arbuscular myeorrhizal fungus in Allium porrum.

S.H. Jabaji-Hare, C.J. Perumalla, and W.B. Kendrick.

INTRODUCTION

This paper reports autofluorescence of vesicles, arbuscules, and hyphae of a Glomus sp. in fresh and cleared root segments of leeks. The component(s) responsible for the autofluorescenee in the VAM fungus are investigated.

MATERIALS AND METHODS

1- A culture of an unnamed species of Glomus (Herb. DAOM 181602), formed vesicles in the host roots, but no external spores. Inoculum production and multiplication was carried out in roots of leeks according to the method of Jabaji-Hare et al. (1984).

2- Segments, single cells of colonized leek root, and isolated vesicles from colonized roots were viewed under a Nikon Apophot epifluorescence microscope with mercury vapour lamp and violet filter assembly (exciter filter transmitting wavelengths from 361-435 nm).

3- Free-hand transverse sections were made, both of colonized and control roots, and viewed using **ultraviolet (UV), violet (V), blue (B) and green (G)** filters having a bandpass of 330-385 nm, 385-42 nm, 420-490 nm, 500-550 nm respectively.

4- Pure chitin powder (0.074 mg mL^{-1}) and vesicles (0.066 mg mL^{-1}) were each suspended in 5.0 mL of Na_2HPO_4 buffer, and their fluorescence spectra were obtained on an SLM 8000 spectrofluorometer.

RESULTS

1- **Figure 1** shows transverse sections of fungal hyphae (**F**), and individual intramatrical vesicles (**VE**) under white light. When the same seetion was observed under UV light, F and VE fluorcsced brightly (**Fig. 2**).

2- **Figure 3** shows an individual colonized cortieal cell with an autofluorescent arbuseule.

3- In addition to the mycorrhizal structures, certain plant elements, such as epidermal walls (E), hypodermis (H), endodermis (**N**), and tracheary elements (X) autofluoresced both in colonized and control leek roots (**Fig. 2**).

4- **UV, V, and B** filters indueed brighter autofluorescenee. The autofluorescence of myeorrhizal structures remained after clearing of transverse sections and whole roots, and under all excitation filters.

5- The emission spectra of both showed identical peaks at **413 nm.** However, when the fluorescence of pure ehitin and vesicles was excited in the same wavelength range, the excitation spectra did not correspond. The excitation spectrum of the vesicles showed two prominent peaks at **305** and **323** nm, while the ehitin displayed a peak at **335 nm.**

DISCUSSION

1- Autofluorescence of VAM structures was seen under four different ranges of wave- lengths. These results **do not agree** with those of Ames et al., (1982) who may havc failed to see the autofluorcscence of VE and H since they examined whole roots.

2- Autofluorescence of plant elements such as in leek roots **is not related to the colonization process of the mycorrhizal fungus, but is due to the presence of suberin in the plant cell walls.**

3- Autofluorescence of VAm structures **persisted even after clearing.** Thus we conclude that the **autofluorescence of Glomus sp. structures is definitely associated with the fungal wall.**

4- The fluorescence spectra of chitin and vesicles. These results indicate that chitin maybe thc main component responsible for autofluorescenee in the fungal wall.

5- The excitation peak of chitin is broad, and it is possible that the peak is somewhat distorted since chitin displays poor solubility in aqueous solutions.

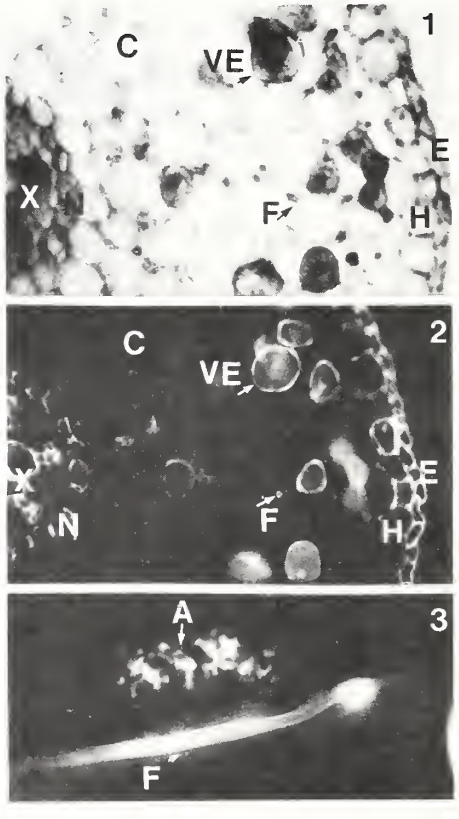


Fig. 1. Transverse section of VAM leek root. e, epidermis;h, hypodermis; ve, vesicles; f, hypha; c, cortex; n, endodermis; x, trachaery elements.
Fig. 2. The same section showing autofluorescence of VAM structures. Epifluorescence optics. X290.
Fig. 3. Autofluorescence of an arbusculr (a). X470. Epifluorescence optics.

MANGANESE AND THE FORMATION OF VESICULAR ARBUSCULAR MYCORRHIZA

by

P.A. McGee

Keywords - Solanum opacum, initial mycorrhizal infection, coarse endophyte, fine endophyte

Introduction

Plants of Solanum opacum were inoculated with VAM fungi and grown in a sandy soil which had been treated with aerated steam. Regardless of whether mycorrhizal inoculum was chopped root pieces or fungal spores, no subsequent VA mycorrhizal infection occurred. All plants were found to have higher levels of Mn in shoot tissue than plants grown in untreated soil. As increased levels of available Mn are often found in soils after heat treatment (Baker, 1970), the effect of Mn on formation of VAM was examined.

Materials and Methods

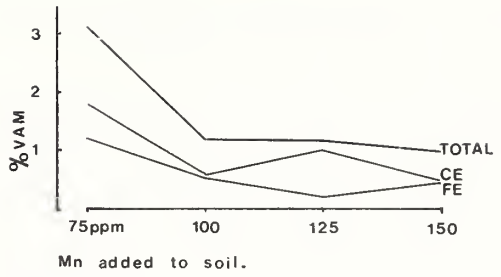
Seedlings of S. opacum were transplanted into pots of the experimental sandy soil amended with 75, 100, 125 or 150 ppm Mn^{2+}, as $MnSO_4$, and harvested after 20, 40 or 60 days growth. Pots harvested at 20 days had 4 seedlings per pot while pots harvested at 40 and 60 days each had one seedling. There were four replicate pots per treatment.

Percentage of root length found to be VA mycorrhizal, percentage of roots infected with coarse hyphaed endophytes (CE) and percentage of roots infected with fine hyphaed endophytes (FE) were calculated at harvest. The concentration of Mn in shoot tissue was also determined.

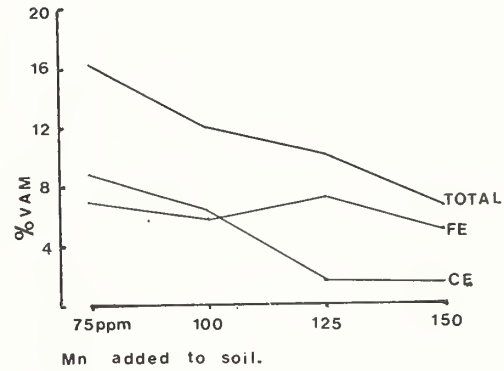
Results and Discussion

At all harvests there was improved root and shoot growth with increasing Mn. At 20 days there was a significant correlation between Mn added to the soil and % total VAM ($r = -0.555$, $p = 0.013$), % CE ($r = -0.531$, $p = 0.017$) and % FE ($r = -0.508$, $p = 0.022$). There was no significant correlation between Mn concentration in shoot tissue and % total VAM, % CE or % FE. At all harvests Mn added to the soil significantly correlated ($p > 0.001$) with Mn in the shoot tissue.

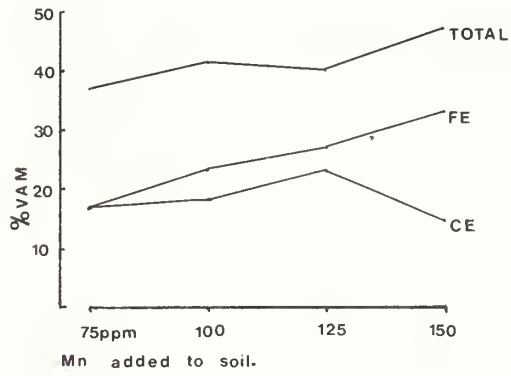
Initial mycorrhizal infection was depressed by increasing levels of Mn in the soil. This may represent reduced spore germination and hyphal growth by increased levels of soil Mn as Hepper and Smith (1976) found Mn over 1360 µg/l in agar reduced spore germination and hyphal growth of Glomus mosseae.



(a) 20 days



(b) 40 days



(c) 60 days

Figure 1. The percentage of roots infected with total VAM, CE and FE, after growth in soil amended with Mn^{2+} and harvested at 20, 40 and 60 days.

In this experiment, Mn was found to affect the development of VAM in the root. FE spread faster than CE in root tissue at higher levels of Mn. The regulation of this growth difference has not yet been investigated.

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Influence of mycorrhizal inoculation, drought and phosphorus on plant growth

By

B. A. Daniels Hetrick, J. A. Hetrick and J. Bloom

Keywords--Glomus mosseae, parasitism, corn

Introduction

Although mycorrhizal fungi benefit plant growth primarily by providing supplemental nutrients these fungi may also improve drought tolerance of colonized plants. However, such drought tolerance may still be related to the phosphorus nutrition of the host (Nelsen and Safir, 1982). Our research examines the influence of phosphorus fertility on the growth and mycorrhizal colonization of corn under well-watered and droughted conditions.

Methods and Materials

Steamed silica sand was amended with 7.5, 15.0, 22.5 and 30.0 ppm KH_2PO_4; a nonamended control was included. Plastic azalea pots (25 cm diam.) were filled with 4.4 Kg of the sand and half were inoculated with 50.0 g Glomus mosseae soil inoculum containing approximately 64 spores/g soil. Pots were seeded with O's Gold corn (SX5500A), watered daily with distilled water to maintain soil moisture of 14-16% (ca. - 0.33 bars), and fertilized weekly with 75 ml of 4X Hoagland's nutrient solution minus phosphorus. After 4 wks half the pots were subjected to drought cycles and watered only after a leaf water potential of -16 bars had been reached. After nine weeks dry weight and percentage root colonization of each plant were determined.

Results and Discussion

Corn grown in sand containing no P or 7.5 ppm P were severely P deficient and no differences between mycorrhizal and nonmycorrhizal plants were evident. At 15 and 22.5 ppm P mycorrhizal corn plants were significantly larger than nonmycorrhizal plants but at 30 ppm P growth of mycorrhizal corn plants was significantly reduced suggesting parasitism by the fungal symbiont. No growth differences were observed between inoculated and noninoculated plants exposed to drought stress at any P level (Table 1). However, mycorrhizal root colonization of droughted plants was significantly greater than well-watered plants at the lowest P level (Table 2). Although mycorrhizal inoculation did not appear to improve growth of droughted plants, consideration of droughted plants as a percentage of well-watered plants revealed a significant benefit from mycorrhizal inoculation at the highest P level (Figure 1). Thus, the high P levels, which reduce mycorrhizal benefit under well-watered conditions, may still be inadequate under severely droughted conditions. At even higher P levels or under less severe drought stress, mycorrhizal improvement of drought tolerance may be more evident.

Table 1. Influence of phosphorus concentration and G. mosseae colonization on the dry weight of droughted and adequately watered corn plants

Phosphorus amendment (ppm)	Dry weight (g)*			
	Adequately watered		Droughted	
	Inoculated	Control	Inoculated	Control
0	1.2^{f}	1.2^{f}	1.6^{f}	1.3^{f}
7.5	28.7^{d}	29.9^{d}	19.5^{e}	19.3^{e}
15.0	50.5^{a}	43.0^{bc}	24.4^{de}	24.5^{de}
22.5	51.5^{a}	42.8^{bc}	26.6^{d}	26.2^{d}
30.0	42.7^{c}	49.2^{ab}	23.8^{de}	24.7^{de}

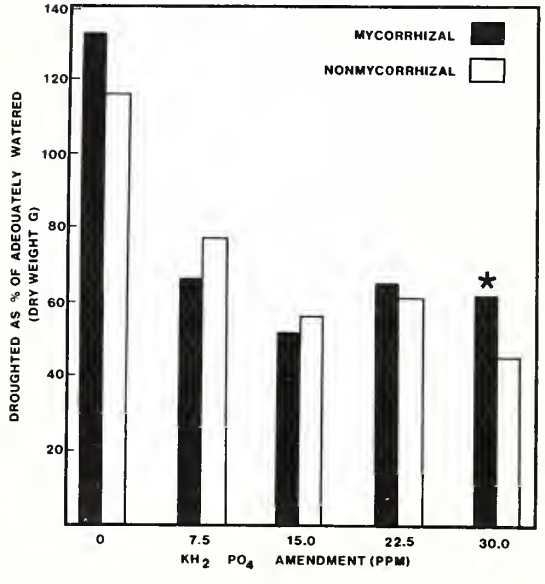
*Means followed by the same letters are not significantly different (P=0.05) as determined by Duncan's multiple range test.

Table 2. Percentage root colonization of corn plants as influenced by phosphorus level and moisture regime

Phosphorus amendment (ppm)	Root colonization (%)*			
	Adequately watered		Droughted	
	Inoculated	Control	Inoculated	Control
0	12.9^{bc}	0^{e}	26.8^{a}	0^{e}
7.5	12.8^{bc}	0^{e}	19.3^{b}	0^{e}
15.0	9.1^{cd}	0^{e}	12.7^{bc}	0^{e}
22.5	8.2^{cd}	0^{e}	7.8^{cd}	0^{e}
30.0	7.8^{cd}	0^{e}	4.3^{de}	0^{e}

*Means followed by the same letters are not significantly different (P=0.05) as determined by Duncan's multiple range test.

Fig. 1. The difference between droughted and adequately watered plants as influenced by mycorrhizal inoculation and soil phosphorus level. Asterisk indicates significant (P=0.05) difference between mycorrhizal and nonmycorrhizal treatments at a given P level.



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PHOSPHORUS NUTRITION ON MYCORRHIZAL COLONIZATION AND PHOTOSYNTHESIS OF *Citrus aurantium* L.

By

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University of Florida, Gainesville, FL

Keywords--*Glomus intraradices*, metabolic sink

Introduction

High P levels in leaf tissue of mycorrhizal host plants has been suggested as a primary reason for high photosynthetic rates and subsequent high growth rates. However, vesicular-arbuscular mycorrhizal (VAM) fungi may also increase photosynthesis by intensification of sink strength or enhanced hormone manufacture.

This study examines the role of P nutrition and VAM colonization on photosynthesis of sour orange seedlings.

Methods and Materials

Sour orange seedlings (*Citrus aurantium* L.) were transplanted at the four leaf stage into 1200 cc plastic containers filled with 1 Canadian peat: 1 fired montmorillinite clay (v/v) medium having 7 mg kg^{-1} available P (bicarbonate-solution analysis). Half the plants were inoculated with the mycorrhizal fungus *Glomus intraradices* Schenck & Smith using a mixture of chlamydospores, hyphae and colonized roots. An inoculum filtrate was applied to roots of non-VAM plants. All plants were fertilized weekly with Hoagland's solution (minus P) and one of four P treatments (0.0, 6.25, 25.0 or 100.0 mg P per 1200 cc container as H_3PO_4) were included in solutions.

Plants were harvested 9 and 26 weeks after inoculation to determine extent of root colonization, P level in leaf tissue and photosynthesis. Photosynthetic rate was measured on the most recently expanded leaves using a plexiglas cuvette in an open flow system through a differential infrared gas analyzer. Photosynthetic photon flux density at leaf surface of 1000 $\mu mol\ m^{-2} s^{-1}$ was provided by a high pressure sodium vapor lamp.

Results and Discussion

Percent VAM colonization was inhibited at the highest P levels 9 weeks after inoculation, but there were no differences 26 weeks after inoculation (Table 1). Plants subjected to higher P fertilization had more sporulation in cortical cells of roots.

Leaf tissue levels of P at 9 and 26 weeks were increased with higher P rates and tissue levels of P were higher with VAM colonization except at 100.0 mg P level (Table 1). Photosynthetic rates (mg $CO_2\ m^{-2} s^{-1}$) were increased from lowest to highest P levels for non-VAM plants, while photosynthetic levels for VAM plants were less affected by P treatment. Photosynthesis was greater in VAM plants at all P levels compared to non-VAM plants except the 100.0 mg rate at 9 weeks.

Table 1. Phosphorus nutrition and VA mycorrhizae (VAM) on photosynthesis determined 9 and 26 weeks after inoculation.

P levels mg per container	Weeks	P (% dry wt.) -VAM	P (% dry wt.) +VAM	Photosynthesis (mg $CO_2 m^{-2} s^{-1}$) -VAM	Photosynthesis (mg $CO_2 m^{-2} s^{-1}$) +VAM
0.00	9	**0.07c	0.20c	**0.21d	0.36d
6.25	9	**0.09c	0.25b	**0.29c	0.38b
25.00	9	**0.21b	0.25b	*0.33b	0.46a
100.00	9	ns0.26a	0.28a	ns0.39a	0.40a
0.00	26	**0.09c	0.13c	**0.19c	0.34b
6.25	26	**0.12bc	0.17b	**0.24b	0.40a
25.00	26	*0.16b	0.19b	**0.26ab	0.40a
100.00	26	ns0.22a	0.24a	*0.30a	0.37a

*,**,ns Significant difference at 5%, 1% level, and not significantly different between -VAM and +VAM plants (Paired t-test).
Means within a column not sharing a common letter differ significantly (5% level) by Duncan's new multiple range test.

Photosynthetic rates were correlated with P content in leaf tissue of control plants but no correlation was observed for VAM infected citrus seedlings (Fig. 1).

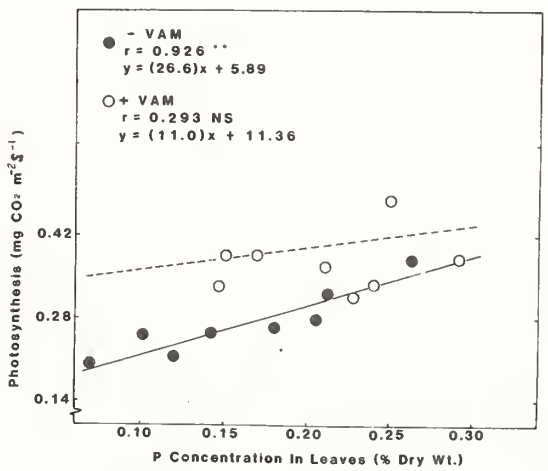


Figure 1. Correlation between P concentration and photosynthetic rate.

This research substantiates that increased P leaf tissue levels, achieved through VAM symbiosis or high P fertilization improves photosynthetic rates and growth of citrus plants. However, P leaf tissue levels are apparently not the limiting factor in elevated photosynthetic rates of VAM colonized vs non-VAM plants. Improved photosynthetic rates as a result of symbiosis may be partially explained on the basis of increased sink strength for photosynthates and/or biosynthesis of hormones that increase photosynthetic rates.

PHOTOSYNTHESIS AND PARTITIONING OF PHOTOSYNTHATE IN VA-MYCORRHIZA, *RHIZOBIUM* AND LEGUME SYMBIOSIS

By

P.Sivaprasad and P.V.Rai

Keywords - *Rhizobium*, *Glomus*, Photosynthate, competition.

Introduction

In tripartite symbiosis, the mycrosymbionts make use of host photosynthate as their carbon source. Hence, the effectiveness of the system depends upon the photosynthetic efficiency of host and the availability of photosynthate to the microsymbionts. A competition between microsymbionts for photosynthate available in the root system cannot be ruled out, since both the microsymbionts are associated with the root system. Photosynthesis and partitioning of photosynthate in *Cajanus cajan* as influenced by dual inoculation of *Rhizobium* and VA-mycorrhizal fungus was studied.

Methods and materials

Cajanus cajan (L) Millsp. cultivar 'Pusa Agethi', VA-mycorrhizal fungus *Glomus fasciculatum* and *Rhizobium* strain IHP 100 were used for the study. Pot experiment in unsterilized P deficient alfisol was conducted with four treatments, viz., no inoculation (M_oR_o), *Rhizobium* alone (M_oR), mycorrhiza alone (MR_o) and dual inoculation (MR) in four replications. Photosynthesis and partitioning of photosynthate was studied with labelled 14Co2. Radio activity present in the leaves (immediately after feeding) and root and nodule (24 hrs after feeding) was considered as indices of photosynthetic efficiency and availability of photosynthate respectively.

Results and Discussion

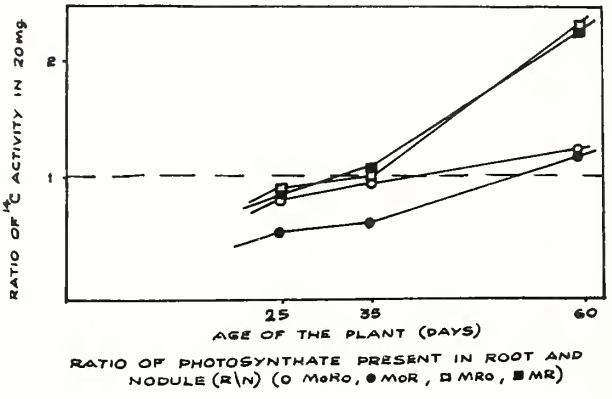
Mycorrhizal redgram leaves consistently recorded an increased 14Co2 fixation. Inoculation of *Rhizobium* further augmented this effect (Table 1). Significant increase in photosynthetic efficiency recorded in tripartite symbiosis on 25th, 35th and 60th day indicates the synergestic interaction of *Rhizobium* strain IHP 100 and VAM fungus *G.fasciculatum* in improving the photosynthetic efficiency of *Cajanus cajan*.

A relationship between mycorrhizal sink and nodule sink was evident from the ratio of photosynthate present in root and nodule (Fig.1). On 35th day, which was a stage of well established mycorrhiza and high nitrogenase activity, the ratio was observed to be around one in mycorrhizal plant. This is a cogent proof of uniform power of rhizobial and mycorrhizal sinks in drawing photosynthate available in the root system. Further, nodule sink of IHP 100 was more powerful than native mycorrhizal sink (ratio-0.61). Weakness of one sink results into the translocation of more photosynthate to the other sink (Fig.1) as noticed on 25th (less mycorrhizal colonisation) and 60th day (senescence of nodule). Hence, there could be a competition between microsymbionts for photosynthate translocated to the root system and under conditions of limited photosynthate availability the tripartite system may result into growth depression. Poor growth and nitrogen fixation in legumes (Sprent, 1973) and growth depression of mycorrhizal plant (Hayman, 1974) observed under shaded condition very well support this view.

Table-1. Photosynthesis in *Rhizobium* and VA-mycorrhiza inculated *Cajanus cajan* 1/

Treatments	14C - activity in leaves ($\times 10^3$ CPM/20 mg) Age of the plant in days		
	25	35	60
M_oR_o	1.68a	3.36a	0.78a
M_oR	1.86ab	4.86b	0.02ab
MR_o	2.34bc	5.52c	1.20bc
M R	2.40c	6.48d	1.32c

1/ Those means within a column not sharing a common letter differ significantly (P = 0.05).



RATIO OF PHOTOSYNTHATE PRESENT IN ROOT AND NODULE (R\N) (○ M_oR_o, ● M_oR, □ MR_o, ■ MR)

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Sprent,J.I.1973. Growth and nitrogen fixation in *Lupinus araboreus* as affected by shading and water supply. New Phytol.72:1005-1022.

HISTOCHEMICAL CHANGES IN PIGEONPEA NODULES DUE TO VA-MYCORRHIZAL ASSOCIATION

BY

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Key words-Rhizobium, Glomus, Cajanus cajan, Bacteroidalzone, polysaccharide

Introduction

VA-mycorrhizal association is known to enhance the nitrogen fixation in legumes. It is understood that N_2 fixation is very much related to nutrient availability, carbon in particular, and number and spread of bacteroids in the nodule tissue. Polysaccharide accumulation and histological changes in nodules of Rhizobium and VA-mycorrhiza inoculated pigeonpea was studied.

Methods and Materials

Experiment was conducted in alfisol with Rhizobium strain IHP 100 and VA^M fungus Glomus fasciculatum. Nodules of 40 days old Cajanus cajan were fixed and embedded in parafin wax. Sections of 5-6 µm thickness were made and were subjected to periodic and schiff test of polysaccharide. Bacteroidal zone(percent),transformed cell size and intensity of polysaccharide accumulation were recorded.

Results and Discussion

Dually inoculated plant recorded 70.06 per cent bacteroidal zone in the nodule tissue which was greater than that of single inoculations (Rhizobium-58.42; mycorrhiza-54.57) and no inoculation(33.67). Further, the size of bacteroid containing transformed cell also enhanced(Fig.1-4) due to mycorrhiza. These changes could be due to the better nutrient availability and/or hormonal effect conferred by mycorrhizal association which might have enhanced the multiplication of both nodule cells and rhizobia. It was evident from the polysaccharide accumulation that Rhizobium (IHP 100) was more effective than native rhizobia in utilizing the carbon (Fig.1 and 2). Mycorrhizal association enhanced the polysaccharide accumulation in nodule tissue (Fig.3). However, it was very less in dual inoculation (Fig.4); probably the synergetic interaction of microsymbionts improved the efficiency of introduced rhizobia and hence better utilization of carbon available in the nodule. Thus, the improved nitrogen fixation in mycorrhizal legumes may be due to the high bacteroid content and effective utilization of carbon available in the nodule.

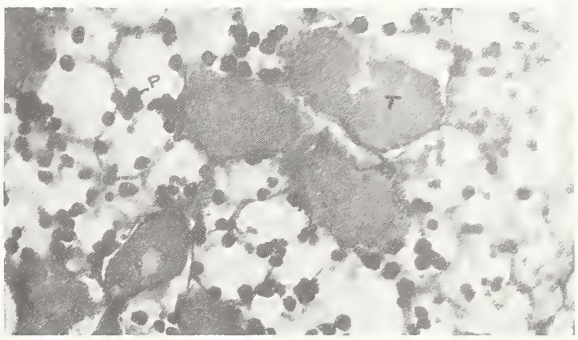

Figure 1. Control (T-bacteroidal transformed cell,P-polysaccharide accumulation) X 400

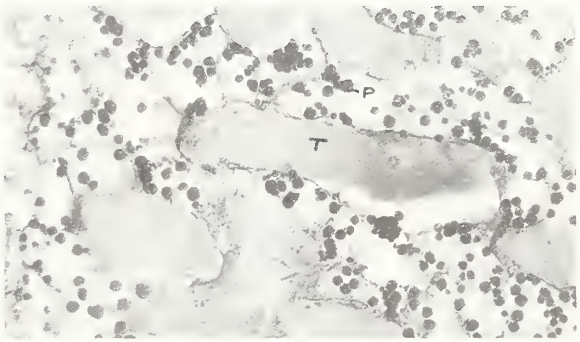

Figure-2. Rhizobium alone inoculation.

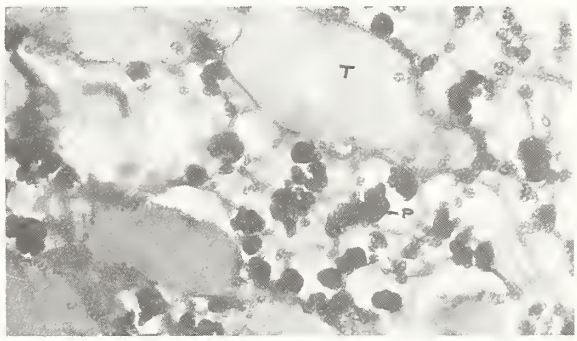

Figure 3. Mycorrhiza alone inoculation.

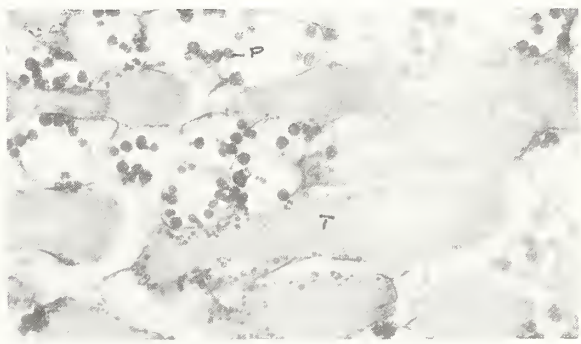

Figure 4. Mycorrhiza + Rhizobium inoculation.

CYTOKININS IN LEAVES OF MYCORRHIZAL CITRUS

By

R. K. Dixon[1], H. E. Garrett[2], G. S. Cox[2]

Keywords--Citrus limon, Glomus fasiculatum, G. mosseae, G. etunicatum, G. epigaeum, G. caledonium

Introduction

Colonization of host root systems by mycorrhizal fungi influences cytokinin synthesis and activity within roots. Allen et al (1980) observed significant increases in root and shoot cytokinin activity of Bouteloua gracilis inoculated with the vesicular-arbuscular (V-A) endophyte Glomus fasiculatum. The influence of mycorrhizal infection on cytokinin activity in the shoots of dicotolydenous plants has not been demonstrated. Hence, the objective of our research was to characterize and quantify cytokinin activity in leaves of axenically grown Citrus limon inoculated with five V-A mycorrhizal symbionts.

Methods and Materials

Citrus limon seedlings were grown in a loamy sand soil containing 30 ppm phosphorus in .5 l pots in a greenhouse. Chlamydospores of the V-A endophytes Glomus fasiculatum, G. mosseae, G. etunicatum, G. epigaeum, and G. caledonium were introduced into the potting medium. Fifteen weeks after germination seedling dry weight, V-A mycorrhizal colonization, phosphorus content and cytokinin content were measured.

Cytokinin extraction from leaf samples was accomplished using procedures described by Horgan and Wareing (1980). Partially purified cytokinin sample extracts were separated and quantified using high pressure liquid chromatography (HPLC) following procedures described by Horgan and Kramer (1979). Definitive identification of cytokinins eluted from HPLC was accomplished using mass spectroscopy (Morris, 1977).

Results

Citrus limon seedlings inoculated with the five V-A endophytes exhibited abundant mycorrhizal development (Table 1). Seedlings inoculated with G. mosseas, G. fasiculatum, and G. caledonium were significantly larger than uninoculated control plants. Leaf phosphorus concentrations were significantly greater in V-A mycorrhizal seedlings.

Inoculation with V-A mycorrhizal fungi increased cytokinin content in the leaves of C. limon seedlings (Table 2). Leaf concentrations of zeatin were significantly greater in seedlings inoculated with G. mosseae and G. fasiculatum compared with uninoculated controls.

Table 1. Total dry weight and foliar phosphorus content of C. limon seedlings inoculated with five V-A mycorrhizal fungi.

VAM fungi	VAM laterals (%)	total dry weight (g)	P (%)
G. mosseae	91a[1]	3.8b	0.17a
G. fasiculatum	94a	4.6a	0.18a
G. etunicatum	84a	1.3d	0.21a
G. epigaeum	81a	1.4d	0.19a
G. caledonium	85a	2.5c	0.12b
Uninoculated control	0b	1.0d	0.12b

[1]Values in each column not followed by a common letter are significantly different (P=0.05).

Table 2. Cytokinin content of leaves from C. limon seedlings inoculated with five V-A mycorrhizal fungi.

VAM fungi	Zeatin (ng/g)	Zeatin riboside ng/g)	dihydro-zeatin (ng/g)
G. mosseae	47a[1]	13a	6a
G. fasiculatum	31b	15a	4ab
G. etunicatum	15c	8a	1b
G. caledonium	20c	7a	5ab
G. epigaeum	11c	8a	1b
Uninoculated control	13c	8a	1b

[1]Values in each row not followed by a common letter are significantly different (P=0.05).

Discussion

The increased cytokinin concentrations in roots and leaves of C. limon may have resulted from fungal cytokinin production and translocation into the plant, inhibition of cytokinin degradation of compounds produced by the fungus or the seedling, or stimulated cytokinin production by the plant as a result of improved nutrition or some other signal from the V-A endophytes. Enhanced cytokinin levels in the V-A mycorrhizal seedlings were correlated with significant increases in total dry weight and improved phosphorus nutrition.

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MYCORRHIZAL SUBSTITUTION OF P REQUIREMENT FOR NITROGEN FIXATION IN RED CLOVER. Jane Yarger and Joseph Morton, Div. of Plant and Soil Sciences, West Virginia Univ., Morgantown, WV 26506.

Key words: Glomus diaphanum, legume, soil solution P, phosphorus nutrition.

Introduction

Red clover forms a dual symbiosis with Rhizobium trifolii and VA mycorrhizal fungi. Mycorrhizae enhance growth, nodulation, and nitrogen fixation in a manner similar to added phosphate (3). We have found red clover on marginal soils, yet nodulation does not occur in sterilized soils with less than 20 mg P kg^{-1} (bicarbonate extraction). Therefore, we sought to quantify the threshold P requirement for nitrogen fixation in mycorrhizal and non-mycorrhizal red clover growing in a pasture soil equilibrated for soil solution P.

Materials and Methods

Soil was collected from a grass-legume pasture containing some volunteer red clover, autoclaved twice, and soil properties determined as follows: paste pH 5.1; 5.7 mg kg^{-1} bicarbonate extractable P; 0.07, 1.3, 0.6, and 0.7 cmol (+p) kg^{-1} K, Ca, Mg, and Al, respectively. A P sorption isotherm was plotted and KH_2PO_4 added to reach pre-determined water soluble P levels (see Table 1). Soil aliquots were incubated at 23% moisture (field capacity) for 65 days to equilibrate solution P.

TABLE 1

Extractable phosphorus concentrations in a pasture soil (Lily series) supplemented with KH_2PO_4.

Supplemental P	Bray-1 extractable P^a	Water soluble P^b
	mg kg^{-1}	
0	6.4	0.031
50	31.4	0.042
75	39.6	0.059

[a] P extracted with .03N NH_4F and 0.25N HCl and determined colorimetrically with Stannous chloride.

[b] P extracted by immiscible displacement of water in ethyl benzyl acetate.

Glomus diaphanum inoculum (as a soil-root-spore mixture) was added to half the soil at each P level and filtrate from the inoculum source was added to the remainder. Soil was added to undraining 750-ml plastic pots, seeded with red clover (6 plants/pot) and inoculated with a liquid suspension of R. trifolii after emergence. Moisture was maintained gravimetrically at 23% throughout the experiment.

Plants were harvested after 6 weeks growth. Nitrogen fixation was measured by acetylene reduction, nodules counted, top and root dry weights obtained, plant N determined by a micro-Keldjahl procedure, and plant P measured colorimetrically after digestion in concentrated H_2SO_4.

Results and Discussion

Soil solution P concentrations did not decrease significantly by the end of the experiment. Thus, the labile pool was large enough to maintain a constant supply of water-soluble P during the growth period.

Dry matter production, nitrogen fixation, and N and P uptake of mycorrhizal and non-mycorrhizal plants were equivalent at 0.02 and 0.06 mg P kg^{-1}, respectively (Figure 1). Biological substitution (2) of inorganic P fertilizer by G. diaphanum was equivalent to 75 mg kg^{-1} (or 150 kg ha^{-1}) in the Lily soil (Table 1). Comparable soil solution P levels in different soils may provide similar growth responses for the same plant species (1). Thus, interactions between dual symbionts and red clover with respect to P fertility in other soils can be compared to the Lily soil only in relation to water-extractable P concentrations.

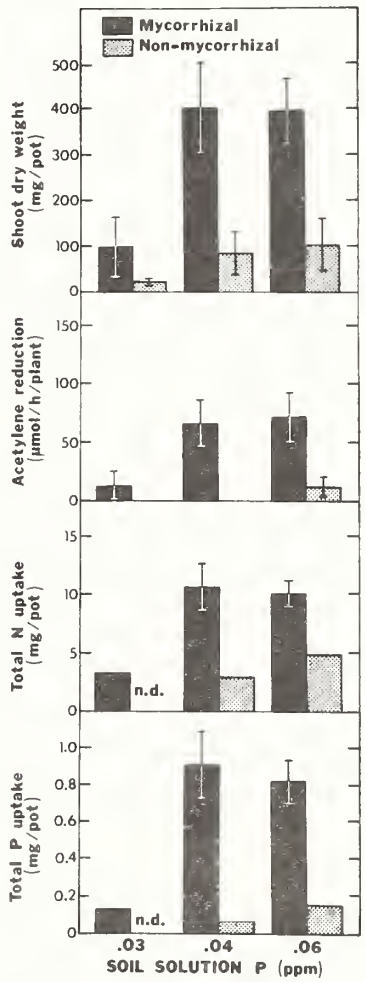

FIGURE 1

The effect of water-soluble P levels on growth, nitrogen fixation, and N and P uptake of mycorrhizal and non-mycorrhizal red clover after 6 weeks growth in an equilibrated pasture soil (Lily series).

Optimum response to mycorrhizal infection occurred with the addition of 50 mg kg^{-1} (100 kg P ha^{-1}). Hence, a minimum water-soluble P level of 0.04 mg kg^{-1} was required for maximum biological utilization of a mycorrhizal fungus.

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MYCORRHIZAE AND SOIL TREATMENT AFFECT ELEMENT CONCENTRATIONS IN ASH SEEDLINGS

by

P.D. Olexia

Keywords--Fraxinus pennsylvanicus, Glomus macrocarpum, nutrients, metals, microbial effects

Introduction

Normal procedures for investigating the role of mycorrhizal fungi in plant nutrition involve growing plants in sterilized soil alone or in sterilized soil supplemented with mycorrhizal inoculum. In this investigation, mycorrhizal infection was achieved in 2 ways: allowing seedlings to become infected with indigenous mycorrhizal fungi in unsterilized soil and through the addition of inoculum to sterilized soil. These two groups were compared to one another and to non-mycorrhizal green ash seedlings on the basis of the concentrations of a number of elements. Soil concentrations of a number of elements also were determined.

Procedures

A heavily weathered loamy soil was collected and air dried (D, below). Some of the soil was sterilized with methyl bromide and added to another 280 pots. A soil/root-fragment inoculum of Glomus macrocarpum was added as a layer near the surface of half of the latter group (C, below). The remaining 140 pots (N, below) contain only sterile soil. Seeds of green ash were added to each of the 420 pots and were covered with soil. Pots were kept in a greenhouse. Seedlings were harvested at 24 weeks and separated into roots and shoots by treatment. Roots were thoroughly rinsed. At harvest, soil samples were removed from pots and air dried at room temperature for 48 hours prior to chemical analysis.

Results

Within 8 weeks most non-mycorrhizal plants had abscised their cotyledons while mycorrhizal plants retained their cotyledons throughout the experiment. Mycorrhizal plants had significantly greater height, leaf surface area, root weight and shoot weight than non-mycorrhizal plants. Table 1 gives concentrations of elements in plant tissues and soils of the different treatment groups.

Reports of higher [N] in nonmycorrhizal plants are confirmed. [Fe^{+3}] tended to resemble those for Al but were not as high, while nonmycorrhizal plants had lower [Mg].

Conclusions

While mycorrhizal shoots had higher [P], nonmycorrhizal roots contained comparatively high [P] suggesting some type of P immobilization. Moreover, sterilized soils had some 25% greater phosphate concentration than unsterilized soil, due, in all probability, to microbial uptake or immobilization.

High [Ca] in the inoculum mixture explains the higher concentrations in inoculated soil samples. However, this difference is not reflected in tissue concentrations. Major differences can be found in shoots of plants grown in sterile vs. non-sterile soil suggesting an important role of soil microorganisms in Ca availability.

Table 1. Element concentrations in tissues of green ash seedlings and soil.

	P§	Ca§	Al*	Mn*	Zn*	Cu*
Shoot						
D	.196	.832	389	60	44.4	44.2
C	.145	.694	261	140	29.8	14.5
N	.120	.643	589	483	52.2	5.1
Root						
D	.231	.231	2445	337	120.5	53.4
C	.166	.220	1408	647	50.4	16.7
N	.202	.187	2977	4062	100.0	18.3
Soil						
D	4.27	156.6	262.8	15.3	2.31	
C	5.74	309.5	236.9	26.1	1.66	
N	5.62	173.9	269.5	35.8	1.59	

§ as % dry wt, * as ppm; D=indigenous inoculum, C=inoculum added to sterile soil, N=nonmycorrhizal

A plausible explanation of the lower concentration of Al in plants to which inoculum had been added may be related to the possible increase in soil pH caused by the addition of Ca resulting in lower Al solubility. Nonmycorrhizal plants have higher Al concentrations in their tissues, especially in the shoots.

Soil [Mn] increased in the absence of soil microorganisms. These differences are reflected in tissue concentrations where nonmycorrhizal shoots have 3.5-8.1x [Mn] and nonmycorrhizal roots have 6.3x-11.9x [Mn].

Soil [Zn] appear to be slightly decreased by sterilization, however major tissue reductions can be seen in plants grown in soil to which inoculum had been added. Such differences may parallel variations in Al concentration via a similar mechanism. Conversely, sterilization results in significant reduction in tissue Cu concentrations, especially in the shoots. Moreover mycorrhizal shoots have 2-8x [Cu] than those of nonmycorrhizal plants.

Potential toxic effects of high Al or Mn concentrations in non-mycorrhizal plants should not be ignored. However, a preliminary study revealed the addition of P enabled non-mycorrhizal plants to grow as well as mycorrhizal plants. Availability of a number of plant nutrients appears to be affected by soil microorganisms other than mycorrhizal fungi. Studies on the affects of mycorrhizal fungi on plant growth and nutrition should take into consideration these effects.

EARLY STAGES IN ROOT COLONIZATION BY VAM FUNGI

By

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Keywords--vesicular-arbuscular mycorrhizae, time-course study, morphology.

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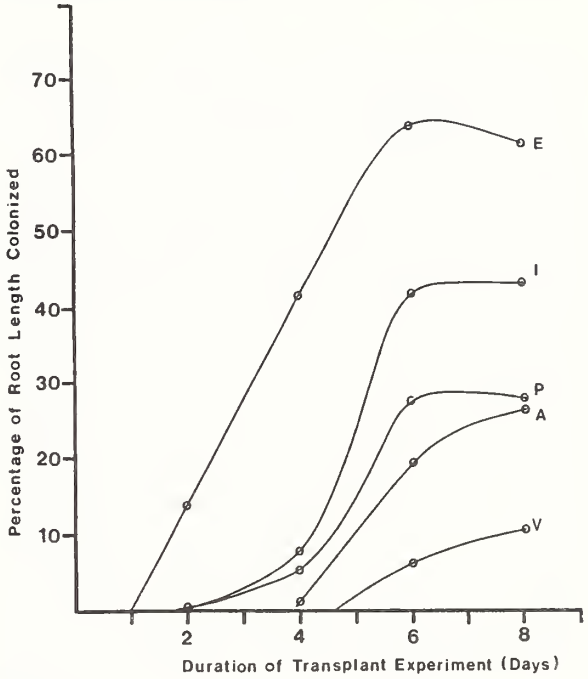
Introduction

A new experimental procedure was developed to produce samples of leek roots containing early stages in colonization by vesicular-arbuscular mycorrhizal (VAM) fungi. This procedure involved transplanting leek seedlings into a pot culture containing an established symbiosis using turface[1] as the growth medium. Abundant colonization of seedling roots occurred in one week. Samples harvested at two day intervals after exposure to the inoculum were used to determine the time-course of colonization. The average distance from growing root apices at which each stage in root colonization first occurred and the growth rate of intercellular hyphae were also estimated. These stages were then documented using the chlorazol black E staining procedure[2]. This transplant procedure should allow the production of abundant material at early stages in VAM development for chemical and morphological investigations as well as providing an efficient inoculation procedure.

Percentage of root length containing external hyphae(E), internal hyphae (I), penetration points (P), arbuscules (A) and vesicles (V) at the two, four, six and eight day harvests.



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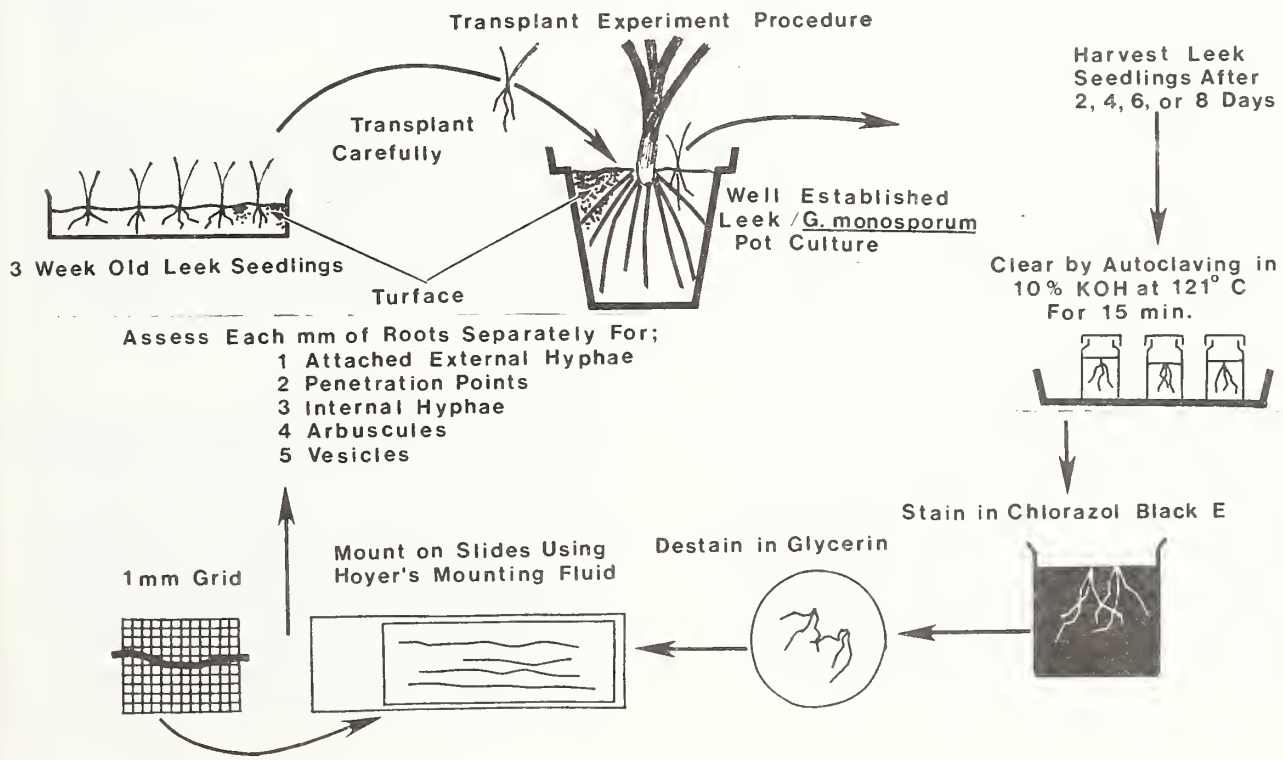

N, P, K, VAM INFECTION, AND GROWTH RESPONSE OF ALFALFA

By

J. Karow and D. Lindsey

Keywords--Medicago sativa, Glomus mosseae, clearing and staining

Introduction

The purposes of this study were to determine the effects of the level of N, P, and K in the growth medium on VAM infection of alfalfa roots and to determine if infected plants have significantly more shoot dry mass than uninfected ones.

Methods and Materials

Alfalfa plants were grown in pots in a lathhouse for 75 days. The experimental unit was the mean value for the 2 plants grown in each pot containing sandy soil. The experimental design was factorial. Nutrient solutions were added once only. Each pot contained either 0.6 or 31 ppm available N, plus either 0.4, 1.5, or 8 ppm available P, plus either 7, 13, or 37 ppm available K for a total of 18 different nutrient combinations. These levels take into account the added nutrients plus the available amount already present in the soil. The two inoculum levels were 0 or 375 Glomus mosseae spores pipetted into each planting hole before sowing. There were six replications per treatment. Na_2SO_4 was used to balance the osmotic potential. The remaining nutrients were those levels used in the Long Ashton nutrient solution (Hewitt and Smith 1974).

A New Staining Procedure

Not one arbuscle was detected in 200 infected roots cleared by autoclaving in 10% KOH before staining. This anomaly required the development of the gentler staining procedure described below. Root samples were placed in 20% glacial acetic acid (GAA) for 10 minutes then in 100% GAA for 15 minutes. After draining, acid fuchsin staining solution (Kormanik and McGraw 1982) was added. After 4 hours at 45C, root segments were placed on a slide and crushed gently. Total infection was recorded using 400 magnification. 25 fields were observed randomly per root. A field was considered infected if its center intersected a mycorrhizal structure. This new staining procedure revealed an average 37% rootlength containing arbuscles.

Results and Discussion

At 0.4 ppm P inoculated plants averaged 42% infection and had significantly greater shoot dry mass than uninfected ones ($P<0.01$). At 1.5 ppm P inoculated plants averaged 39% infection and had significantly greater shoot dry mass than uninfected ones ($P<0.01$). At 8 ppm P inoculated plants averaged 30% infection which was significantly less than at 0.4 ppm P ($P<0.05$) and the shoot dry mass of inoculated plants did not differ significantly from uninfected ones ($P>0.05$).

Table 1. Shoot dry mass of VAM infected compared with uninfected alfalfa and percent infected at 3 phosphate levels.

P In soil (ppm)	VAM Inoculum	Shoot Dry mass (g)	Rootlength Infected (%)
0.4	+	0.17 a	42 x
0.4	-	0.05 b	0
1.5	+	0.37 c	39 xy
1.5	-	0.26 d	0
8.0	+	0.61 e	30 y
8.0	-	0.60 e	0
		*	**

Values in the same column which are not followed by identical letters are significantly different, * $P<0.01$, ** $P<0.05$.

At 0.6 ppm N infection averaged 31% and shoot dry mass of inoculated plants was not significantly different from uninfected ones ($P>0.05$). At 31 ppm N infection averaged 43% which was significantly greater than at the lower N level ($P<0.001$).

Table 2. Shoot dry mass of VAM infected compared with uninfected alfalfa and percent infected at 2 nitrate levels.

N In soil (ppm)	VAM Inoculum	Shoot Dry mass (g)	Rootlength Infected (%)
0.6	+	0.32 a	31 x
0.6	-	0.29 a	0
31	+	0.44 b	43 y
31	-	0.32 a	0
		*	**

Values in the same column which are not followed by identical letters are significantly different, * $P<0.01$, ** $P<0.001$.

No significant N, P, and VAM interaction with shoot dry mass was detected. The K level had no significant effect on shoot dry mass or infection. The results indicated that significant growth response occurred when the P in the growth medium was 0.4 or 1.5 ppm and the N was 31 ppm. The plants with nutrient levels leading to a significant growth response averaged higher infection than those in which there was no significant growth response.

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LEAF RETENTION AND ROOT DEVELOPMENT OF BLACK WALNUT SEEDLINGS IN LOW PHOSPHORUS NURSERY SOILS IMPROVED BY VESICULAR-ARBUSCULAR MYCORRHIZAE

By

Paul P. Kormanik
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Introduction

Premature leaf abscission on black walnut (Juglans nigra L.) seedlings is a common occurrence in forest tree nurseries. Recent reports (Schultz and others 1981, Kormanik and others 1982) indicate that walnut seedlings with VA mycorrhizae grown in soils low in available phosphorus (P) retained their foliage until late fall. Seedlings lacking VA mycorrhizae in low P soil dropped their foliage by late August. Nursery soils are fumigated annually because of the extreme susceptibility of black walnut to root pathogens such as Cylindrocladium and Phytophthora spp. Fumigation also reduces or eliminates the VA mycorrhizal fungal population in the zone of effective fumigation. However, even in fumigated nursery soil (of unknown P levels), some walnut crops retain their foliage until late fall even though their roots may have low VA mycorrhizae colonization. This suggests that some factor in the soil other than mycorrhizae also may be involved in premature leaf abscission of black walnut seedlings.

Methods and Materials

Black walnut seedlings were grown in fumigated soil infested with Gigaspora margarita, Glomus fasciculatum, G. macrocarpum, or in noninfested (control) soil. Three levels of available P (25, 50, and 75 ppm) were imposed on each of these four treatments. The seedlings were grown in 60 microplots (1 X 1 X 0.5 m) filled with approximately 0.25 m^3 of soil on top of a gravel base. Five blocks, each containing three mycorrhizal fungal treatments, a control treatment, and three levels of available P were randomly assigned treatments in a 5 X 4 X 3 factorial design. There were 40 planting locations in each microplot and two black walnuts were planted at each location. When seed germination was complete, seedlings were thinned to one seedling at each planting location.

Leaf retention was observed in late August, approximately one month after discoloration of foliage, in some treatments and in mid-September when leaf abscission was complete in some treatments. Each time, nodes were counted on each seedling and nodes from which the rachis had abscissed were used to calculate percent defoliation.

The study was terminated in early November. For quantification of VA mycorrhizal development, 250 to 500 feeder roots were collected from each of 15 randomly selected seedlings in each microplot and processed using the method described by Kormanik and others (1981). One subjective and one systematic procedure as reported by Kormanik and McGraw (1982) was used to determine the degree of VA mycorrhizal development on the root samples.

Results and Discussion

With 25 and 50 ppm P, VA mycorrhizae significantly improved leaf retention and root weight of all seedlings. At 75 ppm P, seedling development was not affected by mycorrhizal treatment. Within a given mycorrhizal condition, there were only minor differences in growth parameters across P levels. In the nonmycorrhizal treatments, all growth parameters significantly improved at the 75 ppm P treatment, while little difference could be detected between 25 and 50 ppm P. Root collar diameter (cm), height (cm), top weight (g), root weight (g), percent feeder roots colonized, and intensity of VA mycorrhizae infection, respectively, for pooled mycorrhizal seedlings were: 25 ppm P -- 0.85, 46.0, 8.4, 33.0, 49, and 45; 50 ppm P -- 0.85, 46.0, 8.5, 35.2, 49, and 35; 75 ppm P -- 0.85, 47.0, 8.5, 35.2, 44, and 34. The same parameters for nonmycorrhizal seedlings were: 25 ppm -- 0.75, 45.0, 5.9, 22.7, 3.5, and 3.4; 50 ppm P -- 0.77, 46.0, 5.8, 27.0, 0.4, and 1.0; 75 ppm P -- 0.85, 49.0, 8.6, 36.1, 5.7, and 8.4. Seedlings mycorrhizal with G. margarita had more dense root colonization and were larger than seedlings mycorrhizal with either G. fasciculatum, or G. macrocarpum for all P levels.

This research shows that premature leaf abscission can be controlled and root growth improved on black walnut seedlings by providing adequate VA mycorrhizal fungal inoculum in nursery soils over a range of available soil P levels. Comparable seedling development can also be obtained in the absence of VA mycorrhizae if available soil P is maintained at 75 ppm P or higher.

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THE EFFECT OF LIGHT ON MYCORRHIZAL DEVELOPMENT IN SUBTERRANEAN CLOVER

By

M. Tester, F.A. Smith, and S.E. Smith

Keywords -- Glomus mosseae, phosphorus, Trifolium subterraneum

Introduction

Previous work has shown that reduction in light intensity (LI) or duration reduces mycorrhizal infection and the growth response to it (Daft and El-Giahmi, 1978). Alternatively, reductions in growth response may be due to reduced efficiency of phosphate (P) uptake following reduced infection (Hayman, 1974). We report results of preliminary experiments designed to investigate these alternatives.

Methods and Materials

Trifolium subterraneum was grown in a low P soil/sand mix, with inoculum of Glomus mosseae mixed evenly throughout the pots. Plants were grown at LI's of 20, 100 and 450 $\mu E\ m^{-2}s^{-1}$ with a 12 h photoperiod and temperatures of 22°C day/ 15°C night. Plants were harvested at 3 and 6 weeks and plant growth, mycorrhizal infection and P concentration were measured. Mycorrhizal response was calculated from total fresh weight of plants as

$$\frac{\text{FW Mycorrhizal plants - FW non-mycorrhizal plants}}{\text{FW Non-mycorrhizal plants}}$$

Results and Discussion

Reduction in LI decreased % infection and length of infected root per plant (Table 1), as found in previous investigations. Reduction in root : shoot ratio and increase of shoot fresh wt : dry weight ratio indicated that plant growth was limited by reductions in photosynthesis when light was low. At the same time, a tendency for vesicle : arbuscule ratio to decrease suggested that G. mosseae was also carbon limited.

The mycorrhizal growth response tended to increase with increasing LI at both harvests, but the effect was much clearer at 6 weeks than at 3 weeks, despite the fact that, except at the lowest LI plants were well infected by the first harvest (Table 1).

There was no evidence that P uptake limited plant growth at the lowest LI. P concentration in shoots was highest at the lowest LI and progressively reduced as LI increased (Table 2). These concentrations were not high enough to result in P toxicity. The absence of a mycorrhizal effect on P uptake at the lowest LI was to be expected, given the very low infection and number of arbuscules.

We conclude that the reduction in mycorrhizal infection and in growth response at low light is due to carbon limitation and, contrary to Hayman (1974), not to reductions in P uptake.

Table 1. Mycorrhizal infection, root : shoot and fresh weight : dry weight ratios in T. subterraneum at 3 light intensities. 1/

LI ($\mu E\ m^{-2}s^{-1}$)	20		100		450	
Time (weeks)	3	6	3	6	3	6
Percentage Infection	0 (a)	3 (a)	53.5 (b)	61.4 (c)	74.4 (d)	58.8 (bc)
Total Infected Root length (cm)	0 (a)	0.6 (a)	21.4 (b)	156 (c)	86.4 (d)	479 (c)
Vesicle : Arbuscule Ratio (%)	–	–	2.5	1.1	12.9	20.5
Root : Shoot Ratio	0.47 (ab)	0.24 (b)	0.59 (ab)	0.69 (ab)	1.40 (ac)	1.92 (c)
Shoot F.Wt : D.Wt Ratio	15.3 (a)	10.1 (bc)	10.9 (b)	8.2 (bc)	6.3 (d)	7.3 (cd)
Mycorrhizal Response	33	5	49	274	76	577

Table 2. P content of shoots after 6 weeks.

LI ($\mu E\ m^{-2}s^{-1}$)	20		100		450	
± Mycorrhizal plants	-M	+M	-M	+M	-M	+M
P concn (μg P/mg D.Wt)	5.12	4.55	0.77	2.78	0.59	1.26
Total P in shoots (μg)	22.1 (a)	23.8 (a)	26.1 (a)	249 (b)	26.4 (a)	222 (b)

1/ Numbers in the same row followed by different letters are significantly different ($p < 0.05$) by the T method.

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CAN A MYCOPARASITE OF GLOMUS FASCICULATUM SPORES BE A PLANT PATHOGEN?

By

T. C. Paulitz and J. A. Menge

Keywords-- Anguillospora pseudolongissima, Allium cepa, hyperparasite

Introduction

Mycoparasites of vesicular-arbuscular mycorrhizal (VAM) spores have been frequently observed. Some researchers have postulated that mycoparasites might play a role in limiting populations of VAM fungi (Ross and Ruttencutter, 1977; Daniels and Menge, 1980). The purpose of these experiments was to determine if Anguillospora pseudolongissima (A. ps.), shown in vitro to be a mycoparasite of VAM spores, could reduce inoculum potential of VAM, root colonization by VAM, and growth of mycorrhizal plants in the greenhouse. These investigations attempted to determine if a mycoparasite of a VAM spore could act indirectly as a plant pathogen by adversely affecting the fungal member of the mycorrhizal symbiosis.

Methods and Materials

VAM inoculum was prepared from 1½-yr-old pot cultures of Glomus fasciculatum 0-1 (= G. deserticola) on Troyer citrange. The inoculum, consisting of soil, root pieces, and spores, was air-dried for 1 wk prior to incubation with the mycoparasite. A. ps. was isolated from parasitized G. fasciculatum spores extracted from Sudan grass pot cultures. Mycoparasite inoculum was prepared by growing A. ps. on sterile sand + 5% cornmeal for 2 mo.

The experiment had four treatments: (1) Control- no VAM, no mycoparasite,in twice autoclaved blow sand (extractable P (Olsen)= 2-3 ppm). (2) VAM only- no mycoparasite. The air-dried VAM inoculum was serially diluted with sterile blow sand to 10^{-1}, 10^{-2}, and 10^{-3}. (3) VAM + mycoparasite. Mycoparasite inoculum was mixed with diluted VAM inoculum (1 part mycoparasite inoculum : 9 parts diluted VAM inoculum, W/W). The final concentration of VAM inoculum in each dilution was the same as in (2). (4). Mycoparasite only, in the same concentration as (3).

Soil from each treatment was placed in 100 pine seedling tubes and incubated in the greenhouse for 2 wk prior to planting with onion seeds (Allium cepa cultivar Southport Yellow Globe). After 30 d, ten plants were harvested from each treatment every 10 d. The tops were oven dried, and the roots were cleared and stained to determine mycorrhizal colonization. VAM inoculum potential was determined using most probably number (MPN) methods with colonization data from 80, 100, and 110 days.

Results and Discussion

No significant differences in root colonization between mycoparasite and non-mycoparasite treatments were detected in the 10^{-1} and 10^{-2} VAM inoculum dilutions. In the 10^{-3} dilution, significant reductions in colonization were seen in the mycoparasite treatments after 80 days.

A. ps. had similar effects on shoot dry weight. No significant differences were seen in the 10^{-1} and 10^{-2} dilutions. But in the 10^{-3} dilution, onions in the mycoparasite treatments had significantly less shoot dry weights than onions in the non-mycoparasite treatments after 80 days.

MPN calculations showed that A. ps. reduced the VAM inoculum potential in the undiluted inoculum 56.6%, from 23.1 infective propagules/g to 10.0 infective propagules/g.

The effect of the mycoparasite A. ps. on the VAM symbiosis was evident only at lower VAM inoculum potentials. The primary effect of the mycoparasite was to delay ro reduce the incidence of VAM colonization. When VAM inoculum potential is low, the mycoparasite can lower the inoculum potential even further, to the point that a plant can completely escape VAM colonization. If plants are grown in a low P soil, then P deficiencies and decreased dry weights will result. At higher VAM inoculum potentials, this reduction in colonization and dry wieght would not be seen because sufficient numbers of VAM spores would survive to rapidly initiate colonization of roots.

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INOCULUM POTENTIAL--ITS ROLE IN EARLY INFECTION AND MYCORRHIZAL EFFICIENCY

By

J. A. Menge, P. B. Tinker, D. Stribley, and R. Snellgrove

Keywords--Efficiency of mycorrhizae, carbon drain, inoculum potential

Introduction

Mycorrhizal efficiency can be divided into two components--the intrinsic physiological component and inoculum potential. The purpose of this experiment was to determine the relative importance of these two components in the mycorrhizal growth response of leek.

Methods and Materials

Inoculum of Glomus mosseae and Glomus fasciculatum and Glomus constrictum was mixed with a soil at rates of 1:1, 1:10, 1:100 and 1:1000. Ten g of this inoculum was placed under leek (Allium porrum L.) seedlings which were transplanted to pots containing a 2:1 Woburn soil-quartz sand mixture (28 ppm P). Plants were harvested at 12, 18, 26, 36, 46 and 75 days. There were 5 replicates of each fungus-dilution-harvest treatment. At each harvest plants were examined for % mycorrhizal infection, length of internal hyphae/cm root, number of vesicles/cm root, and number of arbuscules/cm root using standard staining and line intersect methods. Dry weights were taken. Inoculum potentials were determined using most probable number methods (Porter, 1979). External hyphae was measured by removing soil and roots from pots and shaking gently in a bucket of warm water. Debris and hyphae associated with the roots after this procedure were rinsed into a sieve with a jet of water, dried, and weighed. P content of shoots was also determined at each harvest.

Carbon metabolism of each mycorrhizal fungus was compared using plants from the 46-day harvest. Plants were selected for similar mycorrhizal infections and P contents. Three plants for each fungus were pulse labelled with 30 μCu $^{14}CO_2$ for 45 min. Plants were monitored for 89 hr and the fate of the photosynthetically-fixed ^{14}C was separated into seven fractions using an apparatus described by Snellgrove et al., 1982.

Results

Inoculum potential and not intrinsic species efficiency appeared to be the major factor which governed the response of leek to mycorrhizal fungi. Inoculum potentials greater than 850 propagules/plant and those less than 17 propagules/plant resulted in significantly reduced leek dry weights regardless of the fungal species involved.

Inoculum potential strongly affected mycorrhizal infection during the first 30 days. Inoculum potentials were inversely proportional to the time of infection, i.e., the length of the lag phase before infection increased logarithmically. Inoculum potentials did not appear to affect either the rate of the logarithmic phase of infection or the infection plateau. Growth responses in leek were generally, but not always, greatest in plants which were infected rapidly.

Growth response in leek due to mycorrhizal infection is mostly closely correlated with the production of external hyphae after 30 days (r=0.948). Other factors affecting the growth response included internal hyphae before 30 days (r=0.910), number of arbuscles before 30 days (r=0.900), % P in the shoot after 30 days (r=0.866), and inoculum potential during the first 30 days (r=0.862).

It appears that time of infection influences the time of maximum external hyphae (r=0.902), which in turn regulates the timing of P uptake (r=0.926). In this experiment P uptake at earlier dates, and P uptake at later dates resulted in smaller growth responses.

Carbon partitioning did not differ significantly among the three fungi. Six to 10% of the total carbon was shifted from the shoot to the roots in all mycorrhizal plants.

Discussion

The amount of external hyphae produced after 30 days appeared to be the primary factor governing growth response in leek. The time of maximum external hyphae regulated the time of maximum P uptake and hence leek growth response.

The amount of external hyphae produced after 30 days was determined by the amount of infection during the first 30 days which was in turn dependent upon the inoculum potential during the first 30 days. It appears that time of maximum P uptake can be regulated by the inoculum potential of mycorrhizal fungi.

The inherent physiology or carbon metabolism of the three mycorrhizal fungi apparently had little to do with the leek growth response.

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PHYSIOLOGICAL CHANGES ACCOMPANYING MYCORRHIZAL INFECTION IN LEEK

By

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Keywords--Allium porrum, Glomus mosseae, phosphorus, relative growth rate, specific leaf area, photosynthesis, time-course.

Introduction

Stribley et al. (1980) pointed out that it is commonly observed that mycorrhizal (M) plants contain higher concentrations of P (dry weight basis) than do non-mycorrhizal (NM) plants of similar dry weight, and that this phenomenom may result from carbon drain by the mycorrhiza. However, there may be a simple explanation related to the delay in increase in uptake of P in mycorrhizal plants: Stribley et al. (1980) quoted data mainly derived from single harvests and in these cases M and NM plants of similar dry weights may have very different relative growth rates and hence %P. The present experiments investigated the time-course of growth and P uptake in M and NM plants.

Methods and Materials

In three experiments leeks (Allium porrum L.) were grown on Y-irradiated (1 Mrad) soil with basal nutrients, with and without the VA mycorrhizal fungus Glomus mosseae, and in a controlled environment (20°C/16°C, 14 h photoperiod):

1) Harvests at 2 and 3d alternately, one level of P, duration 45d
2) Harvests at 10d intervals, six levels of P, duration 60d
3) Harvests at 10d intervals, one level of P for M plants but three levels of P for NM plants to give rates of growth that were similar to and encompassed those of M plants. Duration: 120d.

In a fourth experiment plants were grown in sand culture at three concentrations of P in solution with the liquid in the bed volume replenished at least daily.

Results and Discussion

Rates of accumulation of P and water increased sharply when levels of infection reached c.10% but the increase in dry matter production did not occur until 5-10 days later. These events caused coincident, sharp rises in %P and fresh weight/dry weight ratio but since there was a delay in increase in relative growth rate (RGR), %P and RGR became out of phase for a considerable time. This perturbed %P/RGR relationship may largely account for the observations of Stribley et al. (1980) of unexpectedly high %P in M plants. However, the specific leaf area (SLA: leaf area/leaf dry weight) of mycorrhizal plants in experiment 2 was higher than that of uninfected plants of similar growth rate and remained so for a long time. This may be a mechanism for compeosation for carbon drain since:

$$\text{Relative Growth Rate } \left(\frac{1}{W}.\frac{dW}{dt}\right) =$$

$$\text{Specific Leaf Area } \left(\frac{La}{Lw}\right)$$

$$\times \text{ Unit Leaf Rate } \left(\frac{1}{La}.\frac{dW}{dt}\right)$$

$$\times \text{ Leaf Weight Ratio } \left(\frac{Lw}{W}\right)$$

Experiments 2 and 3 showed that %P, RGR and fresh weight/dry weight reached peaks, the amplitude of which was determined by the P level of the soil, and which thereafter declined. Associated with the initially high levels of %P were high rates of photosynthesis per unit leaf area, which fell as %P declined. The fall in %P was not a result of depletion of soil P since it also occurred in the sand culture experiment where P was constantly replenished. In experiment 3, the initially high values of $\frac{\%P}{RGR}$ for M plants rapidly declined and in old plants a high RGR was maintained at low levels of %P. Here, mycorrhizal plants were very efficient in their use of internal P.

These observations are remarkably similar to the effects of sudden relief of P-deficiency noted in uninfected plants by D. Clarkson et al. (AFRC Letcombe Laboratory UK). The effects of mycorrhizal infection may thus be a simple consequence of delayed relief of P-deficiency and we are devising experiments to test this. Our results strongly imply that it may not be possible to compare any aspect of the physiology of mycorrhizal and non-mycorrhizal plants, and that results from an arbitrary single date of harvest will be very misleading.

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CORRELATION OF GROWTH ENHANCEMENT WITH THE DEVELOPMENT OF EXTERNAL HYPHAE AND ACETYLENE REDUCTION

By

J. L. Kough and R. G. Linderman

Introduction

Vesicular-arbuscular mycorrhizae (VAM) increase plant growth in numerous plant species. A major effect of symbiosis is increased host ability to absorb relatively immobile plant nutrients such as phosphorus, copper, and zinc. Nutrient absorption by the fungal symbiont is theorized to be due to the extra-matrical hyphae of the fungus proliferating beyond the root depletion zone and reaching sources of nutrients otherwise unavailable to the plant. VAM fungi differ in the ability to enhance plant growth even with similar levels of root colonization. These fungi may also differ in production of extra-matrical mycelium (Graham et al., 1982). An indirect assay of external mycelium, sand aggregation by VAM hyphae of VAM fungi (Sutton and Sheppard, 1976), was used to determine if this measure correlated better with plant growth enhancement than the percent root length colonized by VAM.

Acetylene reduction activities in legumes increase with added phosphorus or VAM colonization under conditions of plant growth limited by phosphorus and nitrogen deficiencies. Apparent nitrogen fixation activities in VAM plants increase before any detectable differences in plant growth of VAM plants compared to noninoculated controls. This assay may therefore be a more sensitive method to predict fungus P uptake in legumes than the subsequent, but slower plant growth response. Both acetylene reduction by Rhizobium nodules and sand aggregation by VAM hyphae were used as measurements of the physiological and physical activities of the fungal symbiont as well as the usual parameters of plant growth and nutrient concentrations. Growth enhancement of two legumes (Trifolium subterraneum and Lotus corniculatus) by nine isolates of VAM fungi (Glomus deserticola-GLD; G. epigaeum-GLE; G. mosseae-GLM; G. fasciculatum-GFAB & G624; Acaulospora spinosa-ASP; A. trappei-ATR; Gigaspora gigantea-GIG; G. margarita-GIM) was examined at four harvest times under nonsterile conditions in a glasshouse. In addition, subclover growth enhancement by VAM isolates was determined in a gnotobiotic system.

Methods and Materials

Sterile seedlings of Trifolium subterraneum cv. Mt. Barker and Lotus corniculatus were dipped in a dense suspension (10^6 CFU/ml) of the appropriate Rhizobium strain prior to planting. The gnotobiotic study was done with subclover plants grown in glass tubes with 50 cm^3 of sterile river sand. Both subclover and lotus were subsequently grown in a glasshouse in open pots of increasing size (from 700 to 1400 cm^3) with two plants of the same species per pot separated by nylon screening to prevent root intergrowth and facilitate separation at harvest. VAM fungal inoculum was spores disinfested by 2-week immersion in an antibiotic solution (200 ppm streptomycin and 100 ppm gentiamycin). In both gnotobiotic and glasshouse experiments the final sterile water rinse was collected from the spore treatments and 1 ml of this solution was used per pot or tube to standardize the microflora of the control and VAM treatments. In both studies the VAM treatments were fertilized with 1/4 strength phosphate (11 ppm) Long Ashton's Nutrient Solution (LANS) and the controls with either 1/4 phosphate (11 ppm) LANS or full strength phosphate (43 ppm) LANS, in all cases without nitrogen. The gnotobiotic study was fertilized once after autoclaving. The glasshouse study was fertilized after four weeks growth and biweekly thereafter with 10 ml per pot. The gnotobiotic study was harvested only once at 58 days. Plants were either assayed for acetylene reduction or sand aggregation. In the glasshouse experiments, at each harvest one of the two plants was removed for the acetylene reduction assay. The other plant's top was removed and soil and roots were subsequently dried 4-5 days for the sand aggregation assay (Sutton and Sheppard, 1976). The data were processed by an analysis of variance to determine significant differences and subsequent mean separation was by the Student-Newman-Keul's procedures.

Results and Discussion

VAM and external hyphae formed on lotus but it was not a responsive host to either phosphorus input or VAM colonization. There is an apparent host root physiologic difference between subclover and lotus despite their morphological similarity. Perhaps lotus roots represent an intermediate state of root phosphate physiological development between clovers responsive to VAM and phosphorus inputs and lupines which are highly efficient at phosphorus uptake and not significantly colonized by VAM.

In subclover, the isolates (GLE and ATR) that were superior at plant growth enhancement in the gnotobiotic experiment were also effective in the glasshouse studies. GLE and ATR also had the highest amounts of acetylene reduction activity per gm nodule fresh weight or per plant. The per plant values are a reflection of their larger nodule masses at these harvests. However, in both studies the sand aggregation by ATR did correlate with its growth enhancement activity, but not in GLE. In addition, G624, a G. fasciculatum unable to form extra-matrical hyphae in previous studies, was fully effective with abundant mycelium in this study. This situation indicates a significant influence of host and soil factors involved in VAM effectiveness and again a possible correlation of extra-matrical hyphae and growth response. Some isolates (GIG, GIM and GFAB) had significant VAM colonization, sand aggregation and acetylene reduction without growth enhancement. These results are similar to ineffective Rhizobium nodules and may indicate altered fungal physiology or an unbalanced source-sink relation. The fungus does provide adequate phosphorus but may require excessive photosynthate and thereby prevent growth enhancement.

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EFFECT OF SOIL P, SOIL pH AND VA MYCORRHIZAL FUNGAL SPECIES ON GROWTH OF EXTERNAL HYPHAE

L.K. Abbott and A.D. Robson

Keywords--Glomus fasciculatum, Glomus sp. (WUM 16), Gigaspora calospora, subterranean clover

Introduction

External hyphae of VA mycorrhizal fungi are important for nutrient uptake, stabilizing soil aggregates and spread of infection. Our aim was to test the hypothesis that the length of hyphae formed in soil by VA mycorrhizal fungi relative to the extent of infection was independent of P status of the plant, soil pH and species of fungus.

Methods and Materials

Experiment 1. Steamed Yalanbee gravel (3kg/pot) was used with 6 levels of phosphate. Subterranean clover seeds were inoculated with roots and soil from pot cultures +/- Glomus fasciculatum and harvested after 6 weeks (3 replicates).

Experiment 2. Steamed Lancelin sand (3kg/pot) was used with P adequate for 60% maximum plant growth. Subterranean clover seeds were inoculated with dry soil inoculum of G. fasciculatum, G. calospora or left uninoculated. Plants were harvested after 4, 5 and 7 weeks (3 replicates).

Experiment 3. Steamed Lancelin sand (3kg/pot) was limed to give pH values of 5.0, 5.3, 5.9 and 7.3 (1/5 w/v 0.01M $CaCl_2$). P was adequate for 60% maximum plant growth. Seedlings of subterranean clover were first inoculated with Glomus sp. WUM 16 (in the same soil at pH 7), then transplanted into experimental pots after 4 weeks. Plants were harvested 3 weeks after transplanting (3 replicates).

External hyphae were extracted using the membrane filter technique and its length estimated (Abbott, Robson and de Boer, 1984).

Results and Discussion

Increasing P supply from levels deficient to adequate for plant growth (Fig. 1a) affected the growth of G. fasciculatum in soil and in the root in different ways (Fig. 1b). Increasing superphosphate (10% P) from 0.5 to 1.0g/pot increased the length of external hyphae per cm infected root but decreased the number of vesicles (Fig.1b)

Infection by Glomus sp. WUM 16 spread from existing infection within roots only when external hyphae were formed in soil, i.e. at the highest pH.

G. calospora produced a greater length of external hyphae per cm infected root than did G. fasciculatum (Fig. 2). G. fasciculatum had a higher % infection and formed more hyphae within any section of infected root than did G. calospora (Table 1). The length of external hyphae formed in relation to root length infected changed with time (Fig. 2).

Effects of P supply and soil pH on external hyphae appeared to be closely linked with infectivity.

Differences between G. fasciculatum and G. calospora in the relative extent to which they formed hyphae within the root and in soil provides scope for selecting fungi for inoculation which are able to form large amounts of external hyphae. However, the distribution of the hyphae in soil and their ability to absorb nutrients also need to be considered.

Table 1. Growth and infection of subterranean clover inoculated with G. fasciculatum and G. calospora

Fungus	Fresh wt shoots (g/pot)	% Root length infected	Intensity of infection[1] (% of infected root length in each category)		
			+	++	+++
4 weeks					
G. fasciculatum	6.66a	72	7	26	62
G. calospora	5.21a	35	71	28	0
Nil	5.78a	0			
5 weeks					
G. fasciculatum	17.76d	87	1	11	87
G. calospora	10.98b	55	58	41	2
Nil	13.28c	1			
7 weeks					
G. fasciculatum	24.80f	86	3	37	60
G. calospora	19.39d	57	58	38	3
Nil	22.05e	3			

[1] Intensity categories as proportion of root colonized

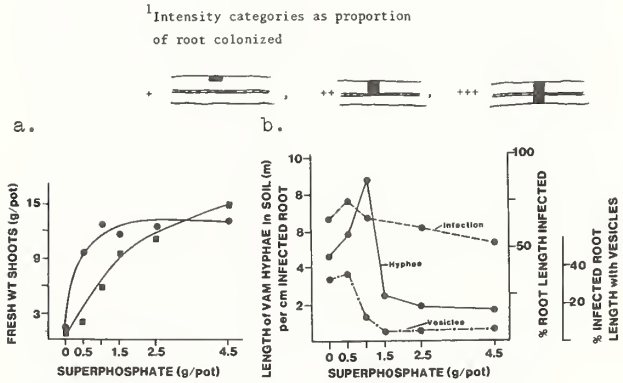

Figure 1. Effect of P supply a) on growth of subterranean clover +(●) and -(■) G. fasciculatum and b) on mycorrhizas formed by G. fasciculatum.

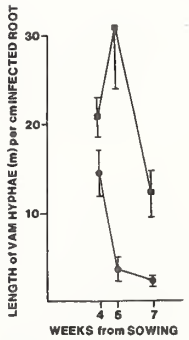

Figure 2. Change in length of external hyphae of G. fasciculatum (●) and G. calospora (■) with time

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SPREAD OF INFECTION BY GLOMUS FASCICULATUM IN ROOTS OF TRIFOLIUM SUBTERRANEUM AND LOLIUM RIDIGUM

M.A. Scheltema, L.K. Abbott, A.D. Robson and G. De'ath

Keywords--Vesicular-arbuscular mycorrhiza, subterranean clover, Wimmera ryegrass, phosphorus, Gompertz curve

Introduction

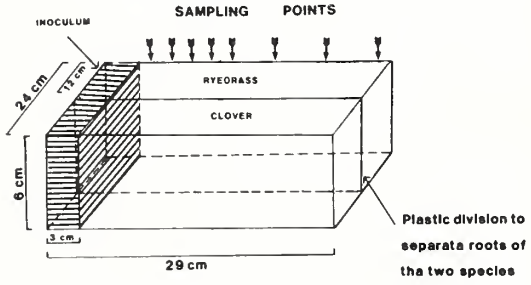
The capacity of inoculant VA mycorrhizal fungi to spread from a localised inoculum is an attribute which needs to be considered when selecting fungi suitable for field inoculation. In our experiment the lateral spread of Glomus fasciculatum from a localised inoculum was examined in clover (T. subterraneum) and ryegrass (L. rigidum). The soil was steamed to eliminate the indigenous VA mycorrhizal fungi so that the ability of the fungus to spread could be studied independently of its ability to compete for infection sites with other VA mycorrhizal fungi.

Methods

The experimental design was a randomised block of a complete factorial of:-
(1) Species of host (clover, ryegrass)
(2) Inoculation (control uninoculated; 800g of a soil/root mix from pot cultures of G.fasciculatum)
(3) 4 harvests (21, 28, 38 & 59 days after sowing)

There were 3 replicates of each treatment. The soil was a lateritic gravel (HCO_3-P (Colwell, 1963) 5mg P g^{-1} soil, pH 5.2 (1/5 w/v 0.01M $CaCl_2$)). Mycorrhizal infection, dry weight and phosphorus concentration of shoots were determined at points 2, 4, 6, 8, 10, 14, 20 and 26 cm from the inoculum.

FIG 1: Pot showing positions of inoculum, sampling points and host species

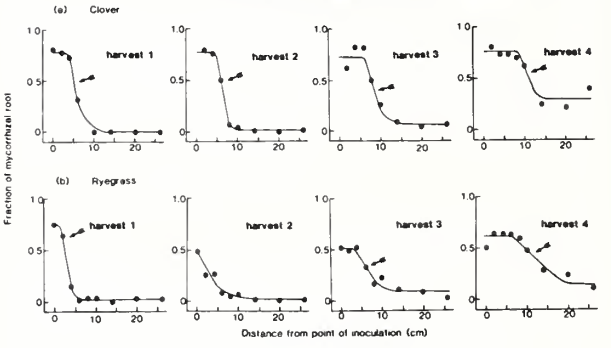


Results and Discussion

1. Plant growth
(a) Inoculation with G. fasciculatum increased the shoot growth of clover but had no effect on the growth of ryegrass, probably because ryegrass maintained a higher P concentration (0.77%) in its tops than did clover (0.17%) in uninoculated plants.
(b) Inoculation had no effect on the P concentration of either plant species.
(c) Root densities of ryegrass and clover were similar.

2. Spread of infection
In both plant species the fraction of root mycorrhizal followed a Gompertz equation (Fig. 2) having:- an upper asymptote, an exponential decline, and a lower plateau of infection.

FIG 2: Effect of Time on the fraction of Mycorrhizal Root at increasing distances from the point of inoculation

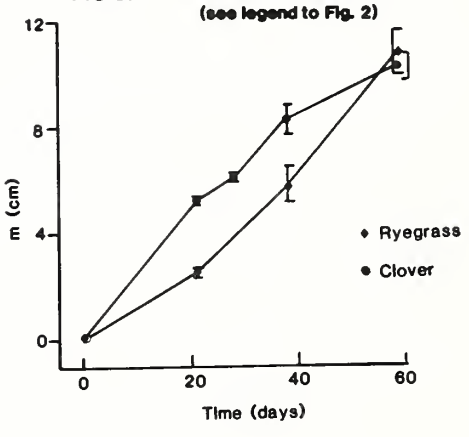


Footnote to Figure 2

1. There was no infection by G. fasciculatum in uninoculated pots.
2. The fraction of root mycorrhizal (y) was best described by:- $y = a+c(\exp(-b(x-m)))$
where a is the upper asymptote, c is the lower asymptote, b is the slope of the exponential decline phase and x is the distance from the inoculum. m (arrowed on graph) is the x value at the midpoint of the curve.

3. Absolute rates of spread
It may not be appropriate to calculate a mean rate of spread over the entire time period as has been done in other experiments (Powell, 1979; Warner and Mosse, 1982) because the rate of spread (dm/dt) (Fig. 3) was dependent on the time period over which the spread of G. fasciculatum was measured.

FIG 3: Effect of Time on "m" (see legend to Fig. 2)



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SULFUR SUPPLY AND INFECTION BY GLOMUS FASCICULATUM

By B.D. Thomson, A.D. Robson and L.K. Abbott

Keywords--Vesicular arbuscular mycorrhizas, subterranean clover, soluble carbohydrates, amino-nitrogen

Introduction

Adding phosphorus may decrease the proportion of root colonized by vesicular arbuscular (VA) mycorrhizal fungi by several mechanisms (Fig. 1).

Because concentrations of both soluble carbohydrates and amino-nitrogen in roots of subterranean clover decrease as phosphorus supply is increased, it is difficult to distinguish between these mechanisms.

Sulfur application increased the concentrations of soluble carbohydrates but decreased the concentrations of amino-nitrogen in lucerne (Rendig and McComb, 1959). We changed the level of sulfur supplied to subterranean clover plants infected with Glomus fasciculatum to determine which of these variables has the greatest affect on VA mycorrhizal infection.

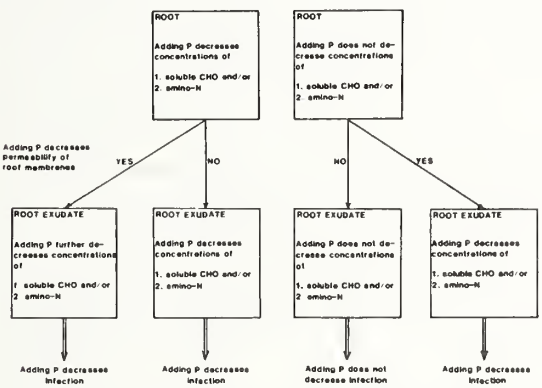

FIG.1 : Possible mechanisms for decreases in VA mycorrhizal infection in response to phosphorus application

Methods

The experimental design was a randomised block of a complete factorial of: (i) 3 levels of sulfur (1.4, 5.7 and 17.1µg of S/g of soil), (ii) ± inoculation with Glomus fasciculatum (100g of roots + soil from pot cultures), with 3 replicates of each treatment.

Steamed siliceous sand was potted into 3kg pots and was supplied with adequate basal nutrients, with phosphorus sufficient for ~75% of maximum plant growth and with the sulfur treatments. Pots were then inoculated and sown with subterranean clover (Trifolium subterraneum). They were harvested 60 days after sowing.

Results

(1) Adding sulfur increased the fresh weight of tops by 40% (Fig. 2). When sulfur was not applied, sulfur concentrations in youngest fully emerged leaves were deficient for plant growth (0.1%) (Fig. 2).

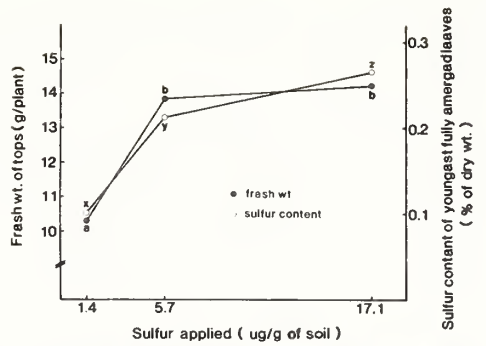

FIG.2 : Effect of sulfur supply on fresh weight of tops and sulfur concentrations of youngest fully emerged leaves . Values with the same letter are not significantly different (p < 0.01).

(2) Adding sulfur did not change the phosphorus concentrations in tops.
(3) Sulfur additions above those required for maximum plant growth decreased the percentage of root length mycorrhizal from 54% to 35%. They also decreased the fresh weight of mycorrhizal root (Fig. 3).
(4) Sulfur additions above those required for maximum plant growth decreased the concentrations of soluble carbohydrates but not the concentrations of amino-nitrogen in roots (Fig. 3).

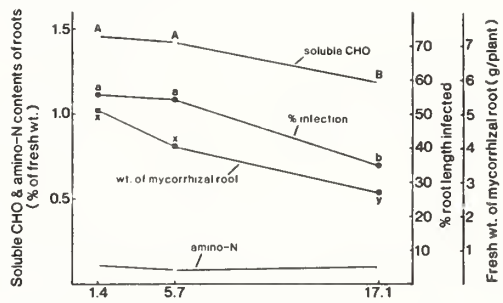

FIG.3 : Effect of sulfur supply on soluble carbohydrate and amino-nitrogen concentrations of roots , percentage of root length infected and fresh weight of mycorrhizal root . Values with the same letter are not significantly different (p < 0.01).

Discussion

VA mycorrhizal infection decreased when sulfur application was above that required for maximum plant growth. This was correlated with decreases in soluble carbohydrate concentrations in roots. It was not correlated with amino-nitrogen concentrations in roots or phosphorus concentrations in tops.

We propose that when sulfur is not deficient for plant growth, the supply of organic carbon is limiting to the VA mycorrhizal fungus and consequently infection is decreased. The inhibition of VA mycorrhizal formation by phosphorus may operate in a similar way.

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VAM INFECTION AND REPRODUCTION AS INFLUENCED BY DIFFERENT ORGANIC AND INORGANIC SUBSTANCES.

By

Manuela Giovannetti and L. Avio

Keywords--Onobrychis viciaefolia, Glomus mosseae, sporocarp production, organic matter.

Introduction

The only way to produce large quantities of mycorrhizal inoculum is the "pot-cultures" technique. A possibility of increasing the efficiency of this system could be an increase in spore production by VAM fungi.

Some authors noted that the production of spores and sporocarps was associated with different organic substances (Koske et al., 1975; Nicolson & Johnston, 1979). Other authors observed a better mycorrhizal infection when soil organic matter or peat were added to soil (Hepper & Warner, 1983). It suggests the possibility of increasing spore production by adding different organic matter to the "pot-cultures".

In the present study the effects of various substances on VAM infection and fungal reproduction were investigated.

Methods and Materials

A sandy soil (35ppm Olsen P, 0.4% organic matter, pH 7.0) was sterilized and mixed with one of the following materials:(1)vermiculite(0.5%), (2)filter paper(0.25%),(3)cellulose powder(0.25%), (4) polyurethan(0.5%), (5) nylon tissue(0.125%),(6) cellulose sponge(0.25%),(7)sponge(0.25),(8)perlite (0.5%), (9)strawberry leaves(0.25%), (10)peat (0.5%). No material was added to control. 4 replicates were used for each treatment. 12-days old sainfoin (Onobrychis viciaefolia, Scop.) seedlings, germinated in sterile sand,were transplanted, one per pot, and inoculated with 30 sporocarps of Glomus mosseae(Nicol. & Gerd.) Gerdemann & Trappe. Plants were grown in a greenhouse (20°C) for 14 weeks.

After harvesting,fresh and dry shoot weights were measured.Roots were stained and VAM infection was determined by the grid-line intersect method. From each pot 100g soil sample was taken and wet-sieved to determine sporocarp number.

Results and Discussion

Sporocarp production in perlite and sponge trials was larger than in control. The other treatments did not produce a number of sporocarps significantly different from the control.Only the cellulose treatments did not produce sporocarps as well as VAM infection, suggesting a negative influence of cellulose on fungal infectivity (Table 1). Differences in soil pH of the various treatments did not influence fungal growth and reproduction since pH values of the different treatments' leachates ranged from 7.5 to 8.2, optimum for G.mosseae growth.

Table 1. Effects of different substances on mycorrhizas and on plant growth.[1/]

Treatment	VAM infection %	Sporocarp N°/100 g a.d. soil	Shoot f.w. mg	Shoot d.w. mg
(1)	42dc	18a	840.0cde	165.2cd
(2)	5a	0	345.0ab	68.2ab
(3)	0a	0	337.5ab	68.4ab
(4)	57bcd	23a	705.0bcd	142.7bd
(5)	44bc	86abc	1267.5ef	232.4de
(6)	3a	0	250.0a	51.6a
(7)	71d	172c	2387.5g	397.7f
(8)	52bcd	159c	1317.5f	268.0e
(9)	39b	10a	540.0abc	119.0abc
(10)	63cd	84abc	1080.0def	199.0cde
C	54bcd	61ab	835.0cde	161.9cd

1/ Those means within a culumn not sharing a common letter differ significantly at the 0.01 level.

The water absorbing capacity of the sponge could have influenced plant growth and sporocarp production, in contrast with the results obtained in peat and vermiculite, which have a similar water absorbing capacity, and also with the results obtained in perlite, a drainage material.

The different nutrient supply of the various substances could have influenced plant growth and sporocarp production. But the largest sporocarp number and the best plant growth were obtained in the treatments which did not supply any nutrient to plants, like perlite and sponge. On the contrary, peat and vermiculite treatments, providing some nutrients, did not differ from control.

In the presence of perlite and sponge a better efficiency of the fungus occurred, that led to a larger development of the plants and to a better fungal reproduction.

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MYCORRHIZAL DEPENDENCY OF SEVERAL PLANT SPECIES UNDER FIELD CONDITIONS IN A SOIL OF MODERATE P. FERTILITY.

By

C. Plenchette, J.A. Fortin and V. Furlan

Keywords--Fumigation, Methyl bromide, Mycorrhizal dependency, Phosphorus, VAM.

Introduction

It was hypothecised by Baylis (1975) that plants with magnolioid root systems are more dependent upon mycorrhizae for phosphorus uptake than plants with graminoïd root systems. The mycorrhizal dependency of plants reflect the degree to which a plant relies upon the mycorrhizal condition to produce its maximum growth or yield at a given level of soil fertility (Gerdemann, 1975). This parameter was numerically defined as the ratio of dry masses of a mycorrhizal plant to a non mycorrhizal one expressed as a percentage (Menge et.al., 1978). Mycorrhizal dependency of plants is obvious in P-poor soil. The purpose of this study was to dertermine the degree of mycorrhizal dependency of several plants species in a soil of moderate P-fertility under field conditions.

Methods and Materials

Different plant species (Table 1) were grown under field conditions in order to compare their development in fumigated and non-fumigated soil. The non-fumigated soil contained numerous propagules of indigenous endomycorrhizal fungi. No phosphorus was added to the soil containing 100 µg/g of available phosphorus (Bray-II) but a basal fertilization of 100 kg/ha $N\text{-}NO_3$ and 100 kg/ha K-KCl was applied on both fumigated and non-fumigated soil. Fumigation was done with methyl bromide at the rate of 45 g/m^2.

A complete randomized block design with four replications was used, each block containing two treatments (fumigated and non-fumigated soil). The experimental units for the 20 plant species were randomly distributed in each half block.

Results and Discussion

All the plants growing in non-fumigated soil synthetised mycorrhizae, except cabbage and garden beet, but different growth responses were observed which allowed to distinguish three groups of plants (Table 1). Plants from group I were heavily stunted in fumigated soil where mycorrhizal fungi had been destroyed. A second group was formed by cereals (oat and wheat) whose growth was identical in non fumigated and fumigated soils. In this case available P-content of the soil (100 µg/g) was high enough to ensure a normal growth for these plants and the mycorrhizae formed in non-fumigated soil were not efficient. Cabbage and garden beet which did'nt formed mycorrhizae belong to a third group. They grew better in fumigated soil where soil-borne pathogens were destroyed and where no competition occur with weeds.

Table 1. Relative field mycorrhizal dependency (RFMD) index for some plant species cultivated in fumigated and non-fumigated soil containing 100 g/g of available phosphorus.

Group	Plant species	$RFMD^{100}$ (%)
I	Tartarian Honeysuckle	91.6
	Common Ninebark	90.2
	Slender Purple-Osier Willow	80.2
	Currant	74.6
	Marigold	73.6
	Purple Leaf Sand Cherry	72.2
	Spirea	69.6
	Carrot	99.2
	Garden Pea	96.7
	Leek	95.7
	Kidney Bean	94.7
	Fababean	93.5
	Sweet Corn	72.7
	Pepper	66.1
	Tomato	59.2
	Potato	41.9
II	Oat	0
	Wheat	0
III	Cabbage	-
	Garden Beet	-

A new method of calculating mycorrhizal dependency is proposed and the calculated value was named relative field mycorrhizal dependency (RFMD) index. It is also proposed that the acronym RFMD receive a superscript representing in µg/g the quantity of available P in the soil.

$$RFMD = \frac{(\text{Dry mass M plant}) - (\text{Dry mass N-M pl.})}{\text{Dry mass M plant}}$$

The results of this experiment shows the importance of VA mycorrhizae since among 20 plant species sixteen had RFMD indices above 40%.

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Plant genotype dependent differences in mycorrhizal colonization rates

By KR Krishna, KG Shetty, PJ Dart and DJ Andrews

Keywords--Colonization, differences, genotype, pearl millet

Introduction

Rapid proliferation of vesicular arbuscular mycorrhiza (VAM) in roots is required for better response. Certain plant species regularly show high VAM colonization while certain grasses rarely attain 50% (Mosse,1980). Of the three components, VAM fungi, plant host and soil environment, information on plant dependent variation in VAM symbiosis and its efficiency is lacking.

Materials and methods

Three field trials were conducted in 1982-83 to examine if VAM colonization was a plant genotype dependent trait. Twenty selected pearl millet genotypes were grown in 3 seperate alfisol soil locations differing in available P status. The trial area consisted of 4 blocks of 20 plots each with 2 rows of 3 m length, spaced 75 cm apart. Treatments were allocated in a RBD. Roots from each plant were carefully dug upto 30 cm depth, collected into plastic bags, cut to 3 cm size and mixed thoroughly. Four random sub-samples for each plant were processed.

Parent vs crosses; we compared the VAM colonization of two selected male-sterile (MS) lines, certain pollen parents and the derived crosses. They were grown in pot filled with natural alfisol soil, pH 6.0 and 4.0 PPM Olsen's P, and harvested after 60 days. There were 3 replicates.

In a pot trial, we made detailed analyses of the relations between the root growth (total root length), the total mycorrhizal root length, and the percentage VAM colonization. Ten genotypes were grown in pots filled with 5 kg alfisol soil (pH 6.5, 1.5 PPM Olsen's P), with and without VAM . The % VAM colonization, the total mycorrhizal root length and the total root length were estimated using gridline intersect method (Geovannetti and Mosse, 1980). There were 6 replicates.

Results and Discussion

The mean VAM colonization of the genotypes, across locations, varied between 25 and 56% (Table 1). Overall, the genotypes tended to show similar rankings for VAM colonization. Attainment of high colonization rates is important. Genotypes showing higher colonization rates are obviously better disposed to harness VAM benefits. The 10 genotypes tested in pots differed significantly for % colonization when challenged with a single isolate, Gigaspora calospora or native VAM flora. Interpretation of % VAM colonization is often confounded by the differential root growth rates (Huisman 1982). Since we found that a cluster of genotypes with similar root length differed for % colonization and total mycorrhizal length, the differences are explicit. Genotype differences could be due to differences in number of root infection sites, differential penetration rates and spread, inturn, dependent on the biochemical and physiological differences of the genotypes.

In the experiment involving parent lines and crosses. The extent of VAM colonization varied widely within each of the three groups (Table 2) confirming the genotypic dependence of VAM colonization.

Table 1. Mycorrhizal colonization of pearl millet genotypes grown in three alfisol soils

Genotype	Origin	VAM Colonization (%)			Mean
		Alf A	Alf B	Alf C	
IP 3277	India	15	11	51	25
IP 3476	India	13	20	49	27
IP 5150	Niger	24	30	35	30
IP 4382	India	15	22	56	31
IP 6045	Niger	21	33	41	32
IP 4807	India	23	28	57	36
IP 5427	Niger	26	27	56	36
IP 5335	Niger	22	33	55	37
WC C 75	India	17	23	73	38
IP 4861	Lebanon	22	26	70	39
ICH 220	India	30	36	52	39
BJ 104	India	27	34	58	40
MBH 110	India	23	30	67	40
IP 3840	India	27	42	52	41
IP 5306	Niger	22	44	70	45
IP 5310	Niger	44	43	61	50
IP 4937	Uganda	30	50	66	48
IP 6538	Mali	33	41	74	50
IP 5140	Niger	37	49	77	54
IP 5921	Senegal	42	59	67	56
	SE	±3.1	±4.4	±4.2	±2.1
	CV%	24	12	14	18

Alfisol A,B,C had 3.5, 5.0, 20 PPM Olsen's P respectively

Although MS line 5141A showed significantly higher VAM colonization than MS line 111A, the hybrids made on 5141A did not necessarily exhibit more VAM colonization than those made on 111A. 111A hybrids were more heterotic positively than 5141A hybrids and low vs low combinations exhibited highest heterosis for VAM colonization. Understanding the inheritance pattern of VAM colonization by the host plant may be worthwhile.

Table 2. Mycorrhizal colonization in pearl millet parents and crosses grown in pots

Genotype	% Colonization
MS lines 5141A	51
111A	37
Crosses - pollen parent	
5141A x 631P-3	51
111A x 631P-3	57 *
631P-3	37
5141A x 733P-1	41
111A x 733P-1	54 *
733P-1	38
5141A x 612P-3	50
111A x 612P-3	50
612P-3	65
5141A x 623P-2	44 *
623P-2	56
5141A x 612P-1	66
111A x 612P-1	54
612P-1	63

CV % = 15; * denotes crosses showing significantly higher/lower coln. compared with either of the parents.

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GROWTH AND PHOSPHORUS UPTAKE RESPONSES TO MYCORRHIZAL INOCULATION IS PLANT GENOTYPE DEPENDENT

By KR Krishna, KG Shetty, PJ Dart and DJ Andrews

Introduction

The efficiency of the mycorrhizal symbiosis depends on the three main factors, plant, fungus and soil. Most of the work has been focused on the isolation and selection of fungi giving higher growth responses. Attempts have also been made to understand the effects of soil factors on mycorrhiza. Information on plant genotypic effects on the growth and phosphorus uptake due to mycorrhizal inoculation is lacking.

Methods and Materials

A pot experiment was conducted using ten selected pearl millet genotypes (IP 5921, 4937, 5140, MBH 110, WC C 75, ICH 220, IP 4861, BJ 104, IP 4382, IP 4807) to examine if they varied for growth response and P uptake due to mycorrhizal inoculation. For each genotype, there were three treatments, uninoculated control in sterilized soil; inoculated unsterilised soil with a single spore isolate of Gigaspora calospora; and non-sterilized soil representing the natural mycorrhizal flora in the soil. The soil contained 1.5 mg P per kg soil extracted with $NaHCO_3$ and pH 6.0. Plants were harvested 60 days after planting. Each treatment was replicated 8 times. Another pot trial examined the response of three West African pearl millet germplasm lines for response to inoculation with Gigaspora calospora in both sterilized and non-sterilized soil. The soil contained 4.5 mg P per kg soil extracted with $NaHCO_3$, pH 6.8 and mycorrhizal fungi of the genera Glomus, Gigaspora, Acaulospora and Sclerocystis, at a total population of 185 spores per 50 ml soil. Each treatment was replicated 4 times. Plant phosphorus contents were measured according to Jackson (1971).

Results and Discussion

The dry matter increases of the ten pearl millet genotypes due to inoculation with Gigaspora calospora in sterilized soil differed significantly. IP 5921 gave maximum response to VAM inoculation. The extent of response to mycorrhiza for BJ 104 was half that recorded for IP 5921 (Fig 1). Hence, under similar soil conditions and inoculation with a single species of mycorrhizal fungus the efficiency of the symbioses is clearly dependent on the plant genotypes. Genotypes, such as ZAN seem to derive a stronger stimulus from the mycorrhizal fungus than others (Table 1). Cultivars of wheat are also known to differ with regard to dry-matter response to mycorrhizal inoculation (Azcon and Ocampo, 1981).

The plant dry-matter is the sum result of many biochemical and physiological processes. Hence the fungal stimulus may have preferentially affected any or a few of these in certain genotypes and not others. Plants with and without mycorrhiza are known to differ in their biochemical composition and physiological functions (Gianinazzi-Pearson and Gianinazzi, 1983). It is possible that the extent of such changes differ with different plant genotype - fungal combinations, in turn leading to genotype difference in dry-matter responses.

Increases in phosphorus uptake by the ten genotypes due to mycorrhiza ranged between nil to 2.5 times

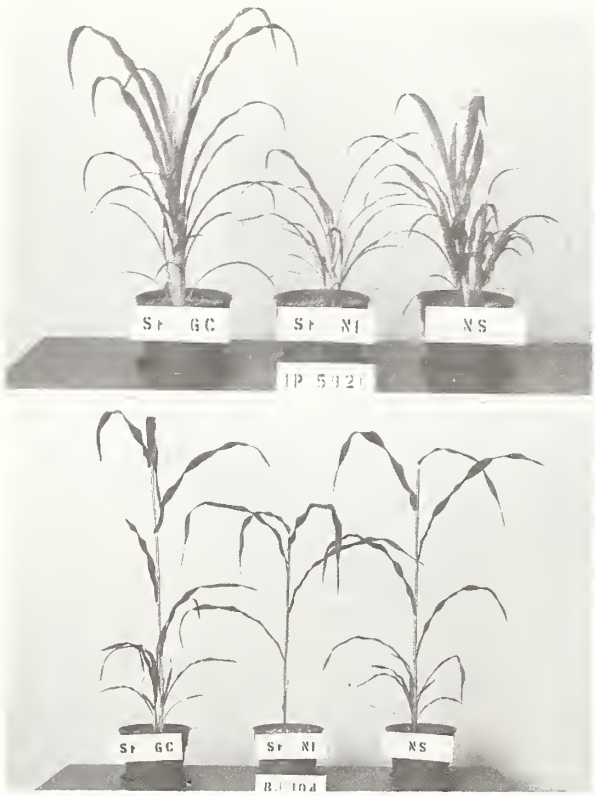

Fig 1. Differential response of two genotypes to mycorrhiza

Table 1. Growth, phosphorus uptake and mycorrhizal colonization of three West African pearl millet genotypes

Soil	Genotype	Shoot dry wt. (g/plant)		P uptake (mg/plant)		Colonization (%)	
		M	NI	M	NI	M	NI
St.	IP-5921	6.91	4.74	16.3	13.0	48^{c}	0
	SDL	4.96	4.20	16.3	13.8	14^{a}	0
	ZAN	7.83	2.93	19.3	8.3	31^{b}	0
Non-st.	IP-5921	5.53	4.73	15.2	14.3	78^{d}	44^{bc}
	SDL	4.19	3.92	11.7	10.2	46^{bc}	35^{bc}
	ZAN	5.47	4.03	12.7	11.0	93^{cd}	52^{cd}
SE		±0.334		±0.81			
CV(%)		20		20		10	

IP-5921=Germplasm line from Senegal; SDL=Sadore local; ZAN=Zanfarwa; M=Inoculated with mycorrhiza - Gigaspora calospora; NI=Non Inoculated
St.=Sterilized; Non-st.=Non sterilized

that of comparable controls. Of the three West Africar pearl millet cultivars, ZAN showed 132% increase in P uptake due to mycorrhiza but the other two cultivars recorded only marginal increases. These results clearly show that increases in phosphorus uptake due to mycorrhizalalso depend on plant genotype. Thus, the VAM activity with regard to P uptake and translocation may be under the control of host genetic constitution and the physiological need for this element. A VAM fungus identified as highly efficient for P uptake on one host, perhaps even a genotype within a crop species, may be ineffective when tested on another host.

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GROWTH AND PHOSPHORUS UPTAKE RESPONSE OF SORGHUM TO MYCORRHIZAL INOCULATION

By

KR Krishna, PJ Dart, KG Papavinasasundaram and KG Shetty

Keywords--Bleeding sap, Glomus, Phosphorus, Sorghum

Introduction

Sorghum is a major cereal crop of the semi-arid tropics and is often cultivated on nutrient deficient soils. Mycorrhizal symbiosis could augment the high phosphorus demand of this crop. However, mycorrhizal role in the P nutrition of sorghum has not received due attention.

Methods and Materials

Five seperate VA fungi, Gigaspora calospora, G. margarita, Glomus fasciculatum, G. mosseae, and Acaulospora were compared with an uninoculated control, on sorghum Sorghum bicolor cv (CSH 5). Plants were grown in sterilized soil:sand (1:1 v/v), having pH 6.7 and Olsen's P 6 mg P/kg soil. Pots, each with a single plant, were placed in a randomized block design in a greenhouse maintained at 26-30°C. Plants were harvested after 54 days. At harvest, bleeding sap was collected by cutting the stem obliquely near the soil surface (Fig. 1) and immediately placing a rubber tubing to fit the size of the stem. The sap was collected for 2 h in the plastic tube, then sucked in to syringe. Shoot dry weights were recorded and phosphorus in the dry-matter and bleeding sap were measured by the vanadomolybdate method (Jackson, 1971).

Results and Discussion

Inoculating sorghum with five seperate VA fungi increased growth 115 to 120%. Growth increase of a similar order have been recorded for pearl millet (Krishna and Dart, 1984) and other crops too. VA fungi varied widely in their ability to stimulate sorghum growth (Table 1). Such a variation in the efficiencies of the fungi could be attributed to differences in P absorption rates of fungal mycelia and their ability to explore more soil area. Plant genotype dependence on mycorrhiza, demand for phosphorus and fungal compatibility could also affect the efficiency of a fungal isolate along with environment (Krishna, et. al. 1984).

Variation in the fungal efficiency necessitates screening for better isolates. Mycorrhizal effects are mainly phosphorus mediated. Phosphorus concentration in bleeding saps of plants treated with VA fungi also differed. This indicates a variation in the amount of phosphorus translocated to the shoot depending on VA fungal inoculum used. Percentage colonization and inorganic phosphorus (Pi) in the bleeding sap was significantly ($p < 0.01$) correlated ($r=0.64$) similarly with drymatter ($r=0.73$). Measuring phosphorus in the stem bleeding sap could be a good indicator for the efficiency of VA fungi for P uptake and translocation. It is also a simple and easy technique.

Table 1. Vesicular Arbuscular Mycorrhizal (VAM) inoculation, plant dry-matter production, and Phosphorus in xylem exudate of sorghum hybrid CSH 5.

Cultures	Percent colonization	Shoot dry matter (g/plant)	Pi in xylem exudate[a] Conc. (ug/ml)
Glomus fasciculatum	66(8.1)[b]	1.93	83
Glomus mosseae	52(7.2)	2.20	59
Gigaspora margarita	48(6.9)	2.07	50
Glomus fasciculatum E3	40(6.3)	1.43	77
Gigaspora calospora	36(6.0)	1.14	28
Acaulospora laevis	32(5.6)	1.33	40
Control	0(0.07)	0.98	20
SE	±(0.32)	±0.15	±5
CV (%)	11	21	21

a Pi = inorganic (or available) P determined by a vanadium molybdate method

b Figures in parentheses are data for per cent colonization analyzed after transformation as x+0.5.

Fig. 1. Bleeding sap collection

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VA mycorrhizal inoculation increases growth and phosphorus uptake of cluster beans

By

N Surender, RS Hari Babu, KR Krishna and B. Gopal Singh

Keywords--Cyamopsis tetragonoloba, Gigaspora, Glomus, Phosphorus uptake

Introduction

Cluster beans are grown in India, both as a vegetable and forage. Being a legume, forms dual symbiosis with both nodulating Rhizobium and the vesicular arbuscular mycorrhiza (VAM). Cluster beans are often grown in sandy soils of very low nutrient status. Its dependence on mycorrhizal symbiosis for nutrients, especially P, can be suspected to be great. Mycorrhizal associations in legumes helps in increasing nodulation and nitrogen fixation (Mosse, 1977). We report our studies on influence of VAM on growth and yield of cluster beans, to which our knowledge is of the first of its kind.

Methods and Materials

The experiment was conducted in pots in green house at APAU Rajendranagar campus with red sandy loam soil of pH 6.3, and P 9.175 mg/kg of soil. Ten VAM isolates obtained from the culture collections of centre of millet microbiology section, International Crops Research Institute for the Semi Arid Tropics, Patancheru, Andhra Pradesh, were used in this study. Approximately 50 g of inoculum containing Ca 1000 extramatrical chlamy dospores was placed below the seeds to ensure that all growing roots passed through the inoculum layer. Five seeds of cluster beans (Cyamopsis tetragonoloba L. (Taub) cv. Pusa navbahar) were sown in each pot of size 20 cm and later thinned to two seedlings after germination. Fertilizer at the rates of 20:60:75 kg NPK/ha in the form of Urea, super phosphate and mu riate of potash were added. The experiment had 12 treatments. There were 10 inoculation treatments, inoculation with fungus 1 (Gigaspora calospora), fungus 2 (Gigaspora margarita), fungus 3 (Glomus mosseae), fungus 4 (Glomus clarum), fungus 5 (Glomus caledonium), fungus 6 (Glomus epigeaum), fungus 7 (Glomus fasciculatum I_1), fungus 8(Glomus fasciculatum I_2), fungus 9 (Glomus fasciculatum I_3), fungus 10 (Acaulospora sp), P check (60 kg/ha) without inoculation and an uninoculated no added P (control). Pots were placed in CRD with four replications, plants were watered to 70% water holding capacity by weight and maintained in the green house for 90 days. Tender pods were harvested at 3 intervals and pooled to complete total yield per plant. Final plant harvest was done 90 days after sowing. The % VAM colonization of the root was determined by the root slide technique after clearing the roots with KOH and staining with trypan blue. Dry weights of shoots were recorded, P content was determined by the Vanodomolybdate phosphoric yellow colour method (Jackson, 1971).

Results and Discussion

Inoculation with VAM resulted in over 70% colonization. fungus 7 (G. fasciculatum I_1) was the most efficient colonizer of the roots. Background VAM population in the control caused 30% colonization and addition of P further reduced it to 20%. The shoot dry weight, nodule dry weight, pod yield and P uptake of VAM inoculated cluster beans was greater than uninoculated control (Table 1) plants receiving P with no inoculation put fourth shoot dry weight nodule dry weight and absorbed P, as much as plants inoculated with VAM. Between them, the VAM differed a great deal in the extent of shoot dry weight respond and this is in confirmity with those reported for soybean (Carling and Brown, 1980). Variation in the fungal efficiency could be attributed to their intrinsic ability to absorb P from the soil and translocate to the plant, ability to explore more soil area, plant fungal compatibility and environment can also influence the symbiotic efficiency.

Table 1. VAM on shoot dry weight, nodule dry weight, yield, P uptake and % colonization of cluster beans

Fungus species	Shoot dry weight (g)	Nodule dry weight (mg per plant)	Pod yield (pods per plant)	Shoot P uptake (mg per plant)	% Colonization
Fungus 1	5.21	16.50	11.50	8.33	78
Fungus 2	5.03	15.75	10.75	7.79	76
Fungus 3	5.32	16.75	11.50	8.70	79
Fungus 4	4.95	15.50	10.50	7.49	72
Fungus 5	4.81	15.00	10.25	7.29	71
Fungus 6	5.17	15.25	11.00	8.18	77
Fungus 7	5.67	17.75	12.25	9.88	87
Fungus 8	5.00	15.75	10.75	7.74	78
Fungus 9	5.16	16.00	11.00	8.16	76
Fungus 10	5.44	15.50	10.50	9.09	75
Phosphorus Without inoculation	5.41	16.00	12.00	9.01	20
Control	3.22	9.00	9.00	3.61	30
CD at P 0.05	0.17	0.87	1.90	1.74	0.06

Significance of VAM are much more in legumes, because of the higher demand for P and the possibility that nodulation and nitrogen fixation can be enhanced. Further studies on the role of VAM on yield increase of cluster beans in field are closely needed, and studies on the significance of VAM in the establishment of cluster beans, in the dry sandy soils and sand dunes in north western India could be interesting.

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Growth and P uptake of Okra as influenced by VAM

By

N Surender, RS Hari Babu, KR Krishna and B Gopal Singh

Keywords--Abelmoschus esculentus, Acaulospora, Gigaspora, Glomus

Introduction

Pot culture experiment with sterilized or unsterilized soils have shown that inoculation with VAM can have marked effects on the growth of a large variety of crops (Hayman, 1982). It was clearly shown that many crops cannot take up sufficient P from low-P soils unless their root become infected with efficient strains of VAM. Inoculation with effective VAM strains can alleviate P deficiency and increase plant growth (Carling and Brown,1980). The present study was conducted to examine the effect of VAM on growth and P nutrition of an important vegetable crop Okra.

Methods and Materials

The experiment was conducted in green house at APAU Rajendranagar campus, with red sandy loam soil of pH 6.3 and P 9.175 mg/kg of soil. Ten different VAM isolates obtained from the culture collections of millet microbiology section, International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Hyderabad were used in this study. Approximately 50 g of inoculum containing ca 1000 extra-matrical chlamydospores was placed below the seeds to ensure that all growing roots passed through the inoculum layer. Five seeds of Okra (Abelmuschus esculentus (L) Moench) cv pusa savani were sown in each pot of size 20 x 22 cm and later thinned to two seedlings after germination. Fertilizer was added at the rate of 44:22:22 kg NPK/ha in the form of urea, super phosphate and muriate of potash. The experiment had 12 treatments. There were 10 inoculation treatments involving VAM viz., fungus 1 (Gigaspora calospora), fungus 2(Gigaspora margarita) fungus 3 (Glomus mosseae), Fungus 4 (Glomus clarum), fungus 5 (Glomus caledonium), fungus 6 (Glomus epigeaum), fungus 7 (Glomus fasciculatum I_1), fungus 8 (Glomus fasciculatum I_2), fungus 9 (Glomus fasciculatum I_3), fungus 10 (Acaulospora sp) a P check without inoculation and an uninoculated control with no added P. The pots were placed in CRD with four replications. Plants were watered to 70% moisture holding capacity by weight and maintained in the green house for 90 days. Tender pods were harvested at 4 intervals and pooled to complete the total yield per plant. Final harvest was done at 90 days after sowing. Percentage VAM colonization of the root was determined by the root slide technique after clearing the roots with KOH and staining with trypan blue. Dry weights of shoots were recorded and P content was determined by the vanadomolybdate phosphoric yellow colour method (Jackson, 1971).

Results and Discussion

Influence of VAM isolates on dry weight, pod yield, P uptake and colonization are shown in table 1. All the fungi significantly (P 0.05) increased plant dry weight and P uptake compared with control but pod yield increased due to inoculation were non-significant. VAM difered in their ability to colonize the root tissue the stimulation of dry matter also differed significantly between the VAM isolates. It ranged between 6% and 22% over control. Fungus 1 (Gigaspora calospora) recorded highest increase in dry matter, pod yield and P uptake. P uptake of VAM plants was equivalent those given P at 22 kg/ha. Okra being a fast growing vegetable crop, P requirements are high, and VAM inoculation may be quite helpful. Similar reports exist for other crops such as soybean (Carling and Brown, 1980, Krishna and Dart 1984).

Table 1. Effect of VAM on growth, yield, P uptake and fungal colonization of Okra

Fungus species	Shoot dry weight (g)	Pod yield (pods per plant)	Shoot P uptake (mg per plant)	% colonization
Fungus 1	7.84	6.50	16.59	64
Fungus 2	7.38	5.75	14.57	60
Fungus 3	6.69	6.00	14.82	62
Fungus 4	6.79	5.25	11.57	54
Fungus 5	7.61	6.00	14.52	60
Fungus 6	6.88	5.50	11.86	55
Fungus 7	7.70	6.00	14.70	61
Fungus 8	7.00	5.50	12.30	56
Fungus 9	7.29	5.75	13.56	58
Fungus 10	7.04	5.50	12.64	56
P control	7.80	5.75	15.99	18
Uninoculated control	6.41	4.50	10.61	23
CD at P 0.05	0.31	1.58	1.39	0.04

Differences in the efficiency of the fungal isolates could be attributed to their intrinsic ability to absorb P from the soil and translocate to the plant, ability to explore more area in the soil. Plant fungal compatibility and environment can also influence the efficiency. Initial pot screening of fungi may help as an initial screening, but field screening to identify effecient VAM seems necessary.

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VAM EFFECTS ON ALFALFA GROWN IN LIMED AND STERILIZED SOIL

by
R. M. N. Kucey

Keywords: Medicago sativa, mixed inoculum, acid soil, long-term effects

Introduction

Although positive responses to VAM inoculation are well established for a wide variety of crops, these studies are mostly concerned with annual crops or initial responses of perennials, and, in many cases, in soils devoid of indigenous VAM. A long-term experiment was performed to study the effects of VAM introduction into sterilized soil on alfalfa growth over nine harvests in the same pot of soil. A second study was designed to view the plant growth response of alfalfa in unsterilized limed acid soil to introduction of VAM adapted to a neutral pH.

Materials and Methods

The VAM inoculum for both experiments consisted of a mixed culture of indigenous VAM isolated from under native vegetation in the SW part of the Canadian prairies and maintained in a mixture of soil and sand at a pH of 7.0-7.4. Roots and adhering soil were used as inoculum.

One-kilo samples of a 1:1 sand:soil mixture were placed in plastic pots for the long-term experiment. VAM inoculum was added to half the pots and three alfalfa seedlings planted per pot. Seven replicates per treatment were used. The plant tops were harvested at flowering for nine successive periods. The shoots were dried, weighed, ground, and analyzed for P content. Roots from root cores were analyzed for VAM colonization and the soil analyzed for available P.

The second experiment utilized an acid soil (pH=5.2) with four levels of added lime as $CaCO_3$ (2.5, 5.0, 12.5, and 50 µg/g) and one level of added P (25 µg P/g). VAM were added to half the pot in each treatment. After 90 days and again after 45 days, the tops were harvested, dried, weighed, ground, and analyzed for P content. The roots were analyzed for VAM colonization.

Results and Discussion

For the long-term experiment with sterilized soil, VAM inoculation resulted in a significant increase for harvest 1 and 3 and a nearly significant increase for harvest 2 and 4 (Fig. 1). Harvests 6, 7, 8, and 9 resulted in VAM plants producing less dry matter than non-VAM plants, however only harvest 8 resulted in a significant decrease. Plant P was only significantly higher for VAM plants in harvest 1 and 3 (Fig. 2). VAM root colonization remained above 80% for the entire experiment.

Growth in limed acid soil was increased by the addition of neutral pH-adapted VAM and by P addition (Fig. 3). Generally, higher soil pH resulted in higher alfalfa dry matter production. Levels of indigenous VAM root colonization decreased with increasing pH, while levels of neutral pH adapted VAM increased with increasing soil pH (Fig. 4).

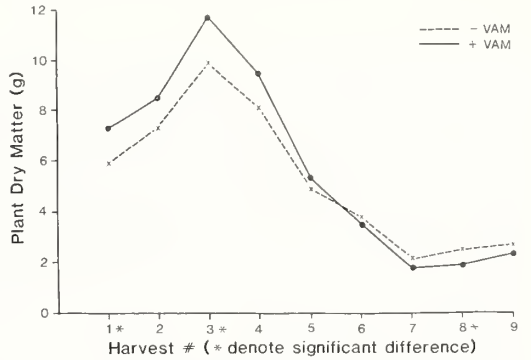

Figure 1. Effect of VAM inoculation on alfalfa growth in sterilized soil.

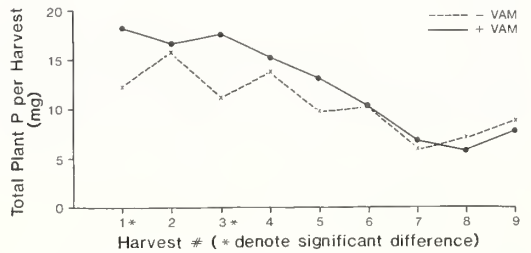

Figure 2. Effect of VAM inoculation on alfalfa P content in sterilized soil.

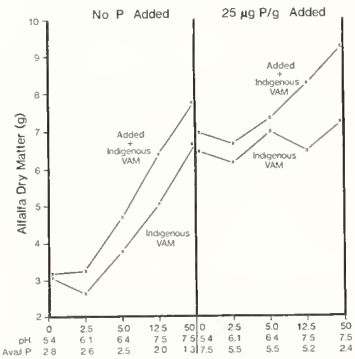

Figure 3. Effect of added VAM, lime, and P on alfalfa growth and soil pH and available P level.

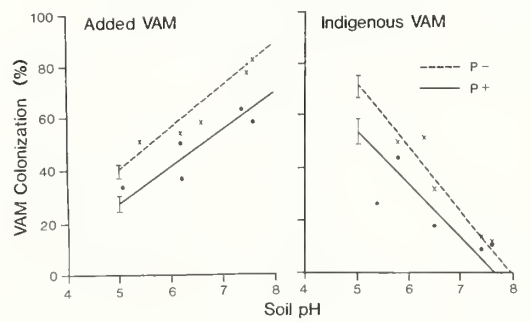

Figure 4. Effect of added lime (soil pH) on levels of added and indigenous VAM root colonization.

INTENSITY OF VAM INFECTION IN COMPOSITAE

By

J. Ardey and K.G. Mukerji

Keywords - VAM, Compositae, Glomus sp.

Introduction

VAM fungi are known to colonize the members of Leguminosae and Gramineae extensively. The present work deals with the formation and intensity of these fungi in Compositae.

Methods and Materials

The plants belonging to Compositae were collected during August 1982 - March 1983 for mycorrhizal examination. Most of the plant species selected were weeds. For all observations the rhizosphere soil was wet sieved and decanted. VAM endophyte invasion into the root tissue was demonstrated by the technique of Phillips & Hayman (1970).

Results and Discussion

In Compositae all the plant species examined were mycorrhizal but the extent of infection varied considerably in each plant species. Percentage infection in decreasing order in the various genera studied was as follows:

Gnaphalium, Bidens, Sonchus, Eclipta, Erigeron, Helianthus and Dahlia (Fig. 1). The last two genera were specifically examined for their dependence on mycorrhiza and it was found that these genera, although mycorrhizal, yet lacked the essential features of the endomycorrhiza, i.e. the vesicles and arbuscules were either absent or poorly developed. It can also be speculated that all the species of VAM fungi do not possess an equal capability to cause infection in host plants, although these may remain, constantly associated with them. As Glomus fuegianum strongly infected Bidens biternata in comparision to G. fasciculatum and Glomus macrocarpum strongly infected Gnaphalium indicum (Fig. 2, 3). The number of spores of Glomus fuegianum & G. macrocarpum were more in the rhizosphere of associated plant species in comparison to spore population of other types. Although, VAM fungal spores predominated in the rhizosphere of the associated plants, yet no positive correlation could be marked out between spore count and intensity of infection. Once the primary infection is initiated the secondary infection is through root to root contact.

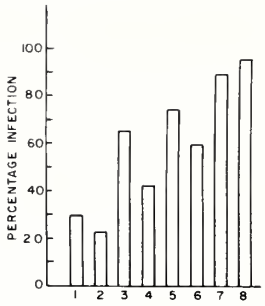

Figure 1. Bar diagram representing the percentage infection in different plant species: 1. Helianthus annus, 2. Dhalia rosea, 3. Eclipta alba, 4. Erigeron canadensis, 5. Sonchus arvensis, 6. Vernonia cinerea, 7. Bidens biternata, 8. Gnaphalium indicum.

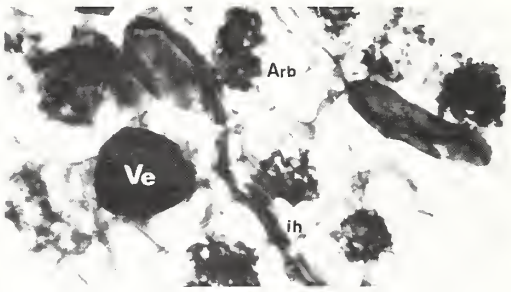

Figure 2. Infection hypha (ih) forming both vesicles (Ve) and arbuscules (Arb) x 260.

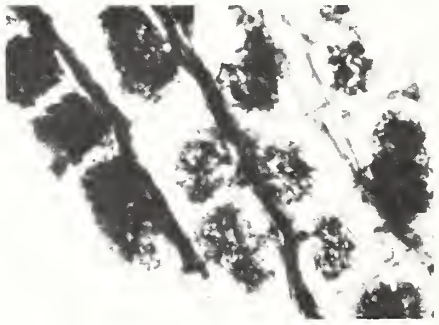

Figure 3. Young arbuscules in the root cortex x 260.

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DEVELOPMENT OF VESICULAR-ARBUSCULAR MYCORRHIZA IN FIELD GROWN BARLEY

By

B. Kochar and K.G. Mukerji

Keywords - Hordeum vulgare, endophyte, VAM, decaying roots.

Introduction

The present work provides information on the chronological establishment and development of vesicular-arbuscular mycorrhiza (VAM) in field grown barley.

Methods and Materials

Barley plants (Hordeum vulgare) were raised in a departmental plot on November 26, 1982 and their roots sampled for VAM infection at regular intervals.

Results and discussion

At the time of first sampling (10 days after sowing the seeds), roots showed little infection. However, few spores of Glomus fasciculatus, Glomus mosseae and Gigaspora sp. were seen germinating on the root surface (Fig. 1 A&B). Internal infection (10-12%) appeared in 60 days old plants. The internal hyphae ran parallel and intercellularly forming H-connections between the parallel hyphae, as are characteristic of Glomus spp. (Fig. 1 C). In 80 days old plants, there was initiation of the formation of arbuscules and the vesicles and the infection increased to 20-30% (Fig. 1 D, E). After flowering, the infection was still increasing (40-50%). The matured 120 days old plants showed maximum percentage infection (65-70%). with vesicles of varying shapes in the root cortex (Fig. 1 F). The high infection level (70%) remained fairly constant as the roots gradually died back. The vesicles developed thick walls to form the resting spores. The decaying roots showed release of thick-walled resting spores that were identical with the chlamydospores present in the soil (Fig. 1G). Here, the VAM formation in barley follows a sigmoid pattern having three phases of development, a lag phase of 40 days at the end of which internal infection started appearing, a phase of fast growth till the plant matured and a static phase when there was no further increase in growth of the fungal endophyte. This pattern resembles those reported in other hosts (Abbott & Robson, 1982; Rao & Parvathi, 1982).

The experimental plot had an average population of 168 spores/10g. soil at the time of sowing the seeds. In the next two months, there was a fall in the number of spores to 123 spores/10g. soil. Thereafter, the spore population increased markedly. A rather sharp increase was observed when the plants had almost senesced (Fig. 2). The characteristic spores present in soil samples were those of Glomus spp., Gigaspora spp., Acaulospora spp. and Sclerocystis spp.

These observations indicate that VAM fungi reproduce in the host roots.

Figure 1 (A-G) Vesicular-arbuscular mycorrhiza in the roots of barley. A. Glomus mosseae spores (S) germinating on the root surface x 150. B. Spore of Gigaspora sp. germinating on the root surface x 175. C. Parallel internal hyphae (Ih) with H-connection x 500. D. Internal hypha cutting off the vesicles (V) x 100. E. Internal hypha with arbuscules (Ar) on its side branches x 300. F. Densely colonized root with vesicles of varying shapes x 180. G. Thick walled resting spores (Rs) in the root cortex x 130.

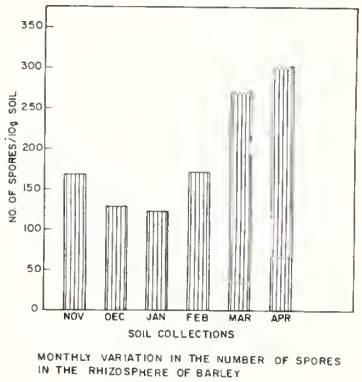

Figure 2

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PROGRESS IN THE DEVELOPMENT OF A CHITIN ASSAY TECHNIQUE FOR MEASURING EXTRARADICAL SOILBORNE MYCELIUM OF V-A MYCORRHIZAL FUNGI

BY: A. G. Jarstfer and R. M. Miller

Keywords: chitin assay, extraradical fungal chitin, soil effects

Introduction

It is recognized that soil microbiology and specifically mycorrhizal research would greatly benefit from a simple quantitative technique for determining fungal biomass in pot cultures or field soils. The technique developed by Tsuji et al. for assaying hexosamines, which was later adapted to plant pathology by Ride and Drysdale, has been used with great success by many to determine intraradical fungal mass as first accomplished by Hepper. Recently Pacovsky and Bethlenfalvay reported use of this colorimetric technique in determining extraradical fungal biomass in the University of California type C potting media. This technique for assaying extraradical mycelia was also used in other experiments by Bethlenfalvay and associates although they noted in their most recent work that use of the assay was not feasible in a "medium textured, red brown, 'Josephine series' soil". Recognizing the complexity of soil, this investigation was to replicate the extraction procedure on various media to which chitin standards had been added.

Materials and Methods

Purified chitin powder (Sigma Chemical, Inc. C-3641) as a suspension in water was used as the standard material. 1.0, 0.5, 0.25 mgs chitin were added to 150 mL Kimax screw-capped tubes containing 25 mL conc. KOH (1.2 G/1 mL H_2O), 25 mL KOH and 25 g washed fine sand (38 µM-300µm), or 25 mL KOH and 25 g washed field soil (38µm-300µm). The field soil used was a coarse loamy, 'Terrada series' soil collected from the Red Desert of southwestern Wyoming. The samples containing KOH and added chitin were autoclaved 30, 60, and 90 minutes at 120°C. Samples containing sand or field soil plus KOH and chitin were autoclaved 60 minutes at 120°C. 4 mL aliquots were removed from each and assayed according to Bethlenfalvay et al. Centrifugation for the samples containing field soil was 5000g for 30 minutes.

Results and Discussion

The autoclaving time of 60 minutes was found to best convert chitin to chitosan whereas increasing autoclaving time to 90 minutes did not increase or decrease the extraction efficiency. The extraction efficiency of the chitosan from the sand media was greater than that reported by Pacovsky and Bethlenfalvay in their sand/perlite media, but much less than in KOH alone (see Fig. 1). The assay procedure for chitin in the field soil failed each time performed. This failure appeared to be an incomplete reaction or a failure of nitrous acid to form in the soil media even though the chitosan had been thoroughly cleaned. The failure of the reactants to become warm upon addition of the nitrous acid and the failure of $NH_4SO_3NH_2$ to react/neutralize the nitrous acid serve as evidence of this failure. The development of the violet color of MBTH-autoxidation also confirms the assumption that the nitrous acid is not neutralized. It is also noted that the solution becomes cloudy and then the violet color disappears. Upon addition of the $FeCl_3$, the solution violently bubbles and a bright orange precipitate forms. All of these problems point to the fact that individual soils may contain physical and/or chemical properties which prevent this technique from working in its present form. Serious work is needed to identify the inhibiting substances which prevent this assay from working in a broader range of soils.

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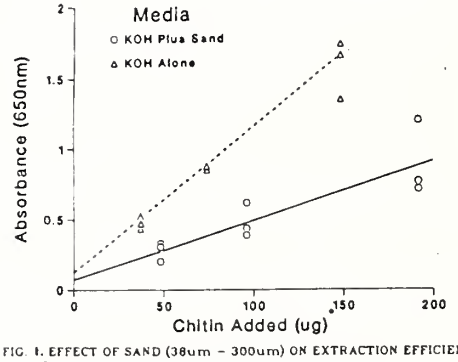

FIG. 1. EFFECT OF SAND (38um - 300um) ON EXTRACTION EFFICIENCY.
No correction for partial degradation as done by Bethlenfalvay et al.
KOH plus Sand - Y = 0.0042 X + 0.073 R = 0.87
KOH Alone - Y = 0.0098 X + 0.126 R = 0.97

INFLUENCE OF VA MYCORRHIZA ON GROWTH, NUTRIENT ABSORPTION AND WATER RELATIONS IN LEUCAENA LEUCOCEPHALA

By

Huang, Ruey-Shyang, W. K. Smith, and R. S. Yost

Keywords--Leucaena leucocephala, Glomus fasciculatus, nutrient absorption, water relations, leaf orientation, drought avoidance.

Introduction

Increased growth and yield of crops due to root colonization by VA mycorrhizae has been attributed to increased uptake of phosphorus (Yost and Fox,1979) and recently to improved water status or transport (Allen et al., 1981). The purpose of this study was to investigate the influence of VA mycorrhiza on growth, nutrient absorption and water relations of leucaena seedlings.

Methods and Materials

Fourteen leucaena seedlings were transplanted in pots containing 4.7 kg fumigated soil (Wahiawa series, Tropeptic Eutrustox). Tha soil pH was 7.4 and the soil P content was 0.078 mg P/l (equilibrated 0.01 M $CaCl_2$ solution). Seedlings were inoculated at planting either with the VA mycorrhizal fungus, Glomus fasciculatus or with fumigated inoculum. Five pots of each treatment received leachings from both the mycorrhizal inoculum and the suspension of Rhizobium sp. Individual pots were weighted to determine the amount of water needed to bring each pot to field capacity. Water relation parameters were measured at 42 and 43 days after germination.

Results and Discussion

All of the growth characteristics and nutrient concentrations listed in Table 1 were significantly higher for mycorrhizal plants than for non-mycorrhizal plants. Mycorrhizal plants had greater shoot and root dry weights, leaf area and root length than non-mycorrhizal plants. Mycorrhizal infection levels of inoculated plants were similar for the two harvests while nodulation increased dramatically.

As shown in Table 1, P and Ca concentrations were much higher in mycorrhizal plants. Because of increased growth of mycorrhizal plants, differences in shoot nutrient uptake were also great. The increased Ca content was though due to increased leaf area and volume of transpirates water rather than due to hyphal uptake.

Plant water relations indicate that mycorrhizal plants had relatively higher stomatal conductance which was associated with higher loss of water and apparently resulted in more carbon assimilation for growth. The higher conductivity associated with mycorrhizal infection is probably responsible for maintaining high xylem water potential despite the low soil water potential in the immediate root zone. However, the stomata of mycorrhizal plants were more sensitive to humidity than those in non-mycorrhizal plants. By contrast, the non-mycorrhizal plants with limited root surface area, absorbed water and nutrients poorly and appeared to conserve water. Thus, non-mycorrhizal plants had lower xylem water potential, stomatal conductance, higher leaf temperature. We have also observed that these plants exhibit more shedding of leaves than the mycorrhizal plants. Consequently, harvest data show that non-mycorrhizal plants were severely stunted.

Table 1. Measured growth characteristics and nutrient contents of mycorrhizal vs. non-mycorrhizal Leucaena leucocephala.

Harvest (days)	49		63	
Treatment	Mycor.	Non-Mycor.	Mycor.	Non-Mycor.
Shoot D. W. (gm)	5.4a*	1.8b	14.5a	1.9b
Root D. W. (gm)	3.0a	1.8b	6.8a	1.9b
Shoot P conc. (%)	0.21a	0.10b	0.17a	0.10b
Shoot Ca conc. (%)	1.25a	0.71b	1.63a	1.28b
Leaf area (cm^2)	899a	182b	1713a	154b
Root length (cm)	10109a	5588b	19122a	6041b
Mycor. infection (%)	83 a	0 b	88 a	0 b
Nodule D. W. (mg)	12.8a	0 a	421.8a	0 b

* Statistical analyses are for individual harvests and different letters denote significant difference (L.S.D. 0.05).

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RESPONSE OF SESBANIA GRANDIFLORA TO INOCULATION OF VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI

By

M. Habte and T. Aziz

Keywords--Glomus fasiculatus, G. mosseae, sterile soil, nonsterile soil, tropical legumes, tropical soils, P-fixing

Introduction

Growth responses resulting from inoculation of crop plants with vesicular-arbuscular mycorrhizal (VAM) fungi are largely explained in terms of increased uptake of phosphorus (Menge, 1983). In the tropics where P fertilizers are expensive and where soils are P-fixing and/or P-deficient, VAM endophytes could play an important role in improving crop production. This is particularly true of the production of tropical legumes which are poor P scavengers owing to their restricted root system and sparse root hairs. With the exception of some Acacia species and Leucaena leucocephala, the VAM affinity of most tropical tree legumes is undetermined. The current investigation is concerned with the interaction of two tropical isolates of Glomus fasiculatus and G. mosseae with the fast-growing tree legume Sesbania grandiflora.

Methods and Materials

Scarified seeds of S. grandiflora were inoculated with Rhizobium and planted in 20.5-cm by 24.5-cm plastic pots containing 6 Kg portions of r-irradiated (2.5 Mrads) or unirradiated soil (Tropeptic Eutrustox) inoculated or uninoculated with a crude culture (infected roots + hyphae + spores) of G. fasiculatus or G. mosseae. Treatments were arranged on greenhouse benches in a RCBD with 5 replicates per treatment. Plants were allowed to grow under natural light for 65 days after which time nodulation, growth, nutrient content and infection level measurements were made.

Results and Discussion

Nodulation, growth and nutrient content measurements showed that the response of S. grandiflora to inoculation was positive and significant in both sterile and nonsterile soil. The data obtained are typical of those summarized in Table 1.

Table 1. Nodulation, dry wt. accumulation and shoot P content of Sesbania inoculated with two VAM fungi.1/

Inoculum	Nodule No.	Plant Dry Wt. (gm)	P Content mg/Plant
	Sterile Soil		
GF	501a	32.0a	29.8a
GM	37b	8.2b	7.9b
None	6c	2.9c	2.9c
	Nonsterile Soil		
GF	184d	19.1d	18.0d
GM	169d	17.1d	17.1d
None	70e	11.9e	11.9e

GF = Glomus fasiculatus GM = Glomus mosseae

1/Means sharing a common letter within a column do not differ significantly at P = 0.05 (Duncan's test).

Response to inoculation of sterile soil with G. fasiculatus, however, was significantly better than that of inoculation with G. mosseae. The endophytes did not differ in their ability to improve growth and nutrient uptake of Sesbania in nonsterile soil. Sesbania infected with G. fasiculatus grew about 2 to 3 times better in sterile soil than in nonsterile soil. On the other hand, the growth of Sesbania in sterile soil inoculated with G. mosseae was less than one-half that in nonsterile and barely comparable or inferior to growth in uninoculated nonsterile soil. The similarity in the effectivity of the two endophytes in nonsterile soil despite the fact that G. fasiculatus was a better colonizer of Sesbania roots (data not shown here) suggests that effectiveness factors such as the extensiveness of external hyphae and their distribution may have been more important than infection levels. The response of Sesbania to inoculation of sterile soil with G. fasiculatus is typical of many VAM-dependent crop plants (Mosse, 1981). The extremely poor performance of G. mosseae in sterile soil as compared to its performance in nonsterile soil, on the other hand, indicates that the activity of the fungus may have been suppressed by toxic ingredients of sterile soil. These results emphasize the limitations associated with the use of sterile soils in screening VAM endophytes.

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ACID-TOLERANT *ACAULOSPORA LAEVIS* INOCULUM FROM A WHEAT FIELD TO GLASSHOUSE -- SURPRISE PROGENY.

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Key words: *Glomus* spp., liming, *Liquidambar styraciflua*, sweetgum

Introduction/Objectives

Prior studies revealed several species of VAM fungi tolerant of high "available" P in cultivated Willamette Valley fields. At one site growing wheat cultivars (Hyslop Farm) on Woodburn silt loam, the type of VAM spores appeared correlated with soil pH. In field 1 (pH 6.7), spores were mostly all *Glomus mosseae* type; in field 3 (pH 5.7), the population was mixed *G. mosseae* and *Acaulospora laevis* plus a few other small *Glomus*-type spores; in field 5 (pH 4.9), recovered spores were all *A. laevis* except for an occasional *G. mosseae* or small *clarum/occultum*-type and an occasional small *Sclerocystis* sporocarp.

Objectives of this study were to test over a range of pH levels, under controlled glasshouse conditions the performance of intriguing acid- and P- tolerant *A. laevis* from field 5, and the potential shift toward larger numbers/proportions of *G. mosseae* (or other) spores with progressive rise in soil pH.

Methods and Materials

Glasshouse trials; separate 20 wk. experiment with 2 hosts, sudangrass and sweetgum (*Liquidambar styraciflua*). Treatments included:

2 soils: P deficient/fixing Jory clay loam; P sufficient Woodburn silt loam (80 ppm); both strongly acid @ pH 4.9 @ collection (see Table 1 for nutrient levels).
x 5 pH levels/soil: limed from 5.0 to 7.7 with powdered $CaCO_3$ and equilibrated 4 wks.
x 2 VAM conditions: live vs. autoclaved inoculum of spores, soil & wheat-root fragments; mean spore count = 82 *A. laevis*/100g soil
x 7 plants/treatment x 3 replications per above

Bulk quantities of both soils were diluted 1:1 with river sand, portions appropriately limed, steam pasteurized, and potted in 155-cc "Super-cells". A 4.5-cm layer of inoculum was banded 1 cm beneath seeds. VAM colonization was monitored periodically. At harvest, growth parameters were measured, % of root length colonized determined, and soils sieved for spore counts and observations.

Table 1. Values @ pH extremes of soil:sand mix.

Soil	pH	P	K	Ca	Mg	CEC	O.M.
		ppm	ppm	(----meq/100g----)			%
Jory	5.2	2	148	4.6	2.7	13.0	0.27
Woodburn	5.5	49	222	4.4	1.2	10.5	1.40
Jory	7.7	1	156	22.3	1.9	--	--
Woodburn	7.7	48	230	11.1	1.0	--	--

Results and Discussion

A. laevis colonizations of the first experiment host, sudangrass, were few and scattered; there were no significant growth responses or consistent correlations with pH--contrary to expectations. (Unmet spore aging requirement? Tommerup, 1983). Appreciable colonizations that varied with pH did occur with the 2nd host, sweetgum (Fig. 1); and obvious significant growth responses to the colonizations did develop (Fig. 2).

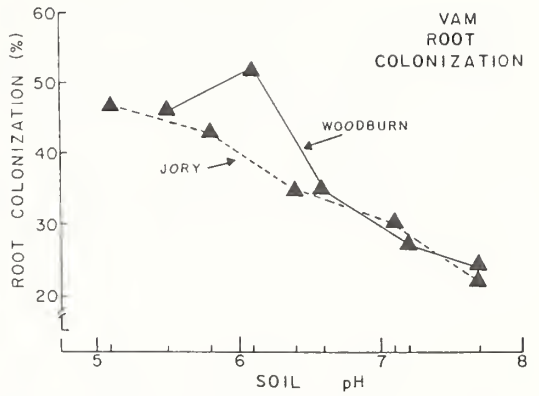

Figure 1. Liming influences on VAM root colonization.

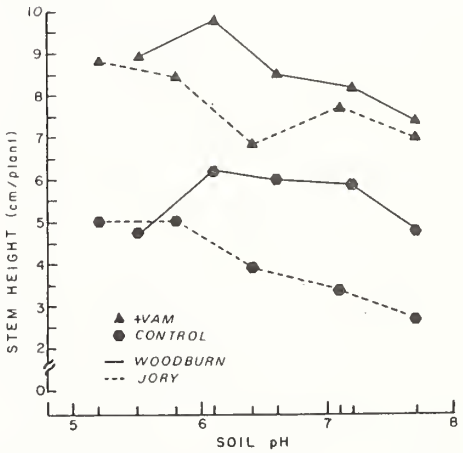

Figure 2. Sweetgum growth responses @ pH level.

Nature of the dominating/operative endophyte came as a huge surprise, however. At final harvest. signs of multiple-species colonizations became apparent. Hundreds to thousands of small, hyaline spores--often clustered as sporocarps to segments of VAM-sweetgum roots--were uncharacteristic of either *A. laevis* or *G. mosseae*. Nearest match to described species suggested *Glomus pallidum* (Hall, 1979). Expected *A. laevis* spores appeared in soils in some treatments, but were generally few and inconsistently scattered among reps. Which of the fungal species actually stimulated the growth response remains uncertain, as do the specific causes for the dramatic change in spore population type. Although *G. pallidum*-like spores were not detected in the field-soil material used as inoculum, viable propagules could have been present in other unrecognized form, e.g., vesicles/hyphae, spores within spores.

T.-H. Message

Confirmation of the species occurring at the end, as well as the beginning of experiments with VAM fungi appears desirable, if not essential. Despite the apparent purity of one's culture, reliance solely on identified spores from mixed soil-root inoculum offers promise of mistaken identities, and misattributions as to the causal organism, i.e., possible serious misinterpretations.

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EFFECTS OF VA MYCORRHIZAL FUNGI ON THE GROWTH OF SUNN CROTALARIA

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Keywords-- Crotalaria juncea, Sudangrass, Glomus, Rhizobium, Phosphorus.

Introduction

Sunn crotalaria (Crotalaria juncea L.) is one of the important green manure crops in the central part of China. It is used for sowing between the rows of 1-2 year plantations to stimulate the growth of young trees. On its roots have been naturally colonized VA mycorrhizal fungi as well as Rhizobium. Phosphorus concentration in soils greatly influenced the development of the VA mycorrhizal fungi and nodulations, and eventually the growth of the hostplants. The purpose of this study was to determine the effects of VA mycorrhizal fungi, nodules and concentration of phosphorus application on the growth of sunn crotalaria.

Methods and Materials

In infection experiment, 6 different species of VA mycorrhizal fungi were used: Glomus sp. No 1 was isolated from the soil Bunxe Plantation of Province Shandong; G. epigaeus and G. sp. No 2 were obtained from Prof. V. Furlan and J. A. Fortin, Canada; G. mosseae, G. fasciculatus and G. sp. No 3 were obtained from the Academy of Agriculture. In phosphorus experiment, there were 10 treatments: seedlings inoculated and non-inoculated with VA mycorrhizal fungi were applied with phosphate fertillizer (14% P_2O_5) at 5 different levels (0, 0.5, 2, 4 and 8g per pot). In experiment of inoculation with VA mycorrhizal fungi and Rhizobium, there were 8 treatments: seedlings of sunn crotalaria were inoculated simply with G. epigaeus, or simply with Rhizobium, or both Glomus and Rhizobium, or were not inoculated at all; in soils either applied or unapplied with phosphorus. Nodulation was formed by inoculating the roots of seedlings with 1 ml suspension of Rhizobium retaining for about 30 minutes. G. epigaeus, as the inocula, multiplied and carried on the roots of sudangrass. The inoculum of 50g of colonized root segments was spread 5 cm below the soil surface in the pot, into which 5 seedlings with 2 cotyledonous leaves were transplanted. Each treatment established 4 replicates. Pots were ramdonized in the greenhouse for 90 days. At harvest, nodules were assessed using the method of Trinick, M. J. (1976). Roots were stained by boiling for 20-30 minutes in 0.05% acid fuchsin in lactoglycerol. In each treatment foliage was chemically analysed. Statistical analysis was done by LSD and q tests for height, dry matter and foliage analysis.

Results and Discussion

Among the 6 different species of VA mycorrhizal fungi used in infection experiment, G. epigaeus and G. sp. No 2 obtained from Canada gave the highest percentage of root colonization (97 and 100 % respectively). G. fasciculatus didn't affect the roots of sunn crotalaria at all. Glomus sp. No 1 isolated from the soils of original plantation had relatively little affection (25 %). In experiment with application of different levels of phosphorus, at harvest, that is, 90 days after sowing, the plants applied with 4g phosphates per pot and simultaneously inoculated with G. epigaeus showed the highest growth and produced the most biomass. The highest amount of Phosphates didn't increase the growth of inoculated plants, but even decreased it in the non-inoculated treatments.

Plants inoculated with VA mycorrhizal fungi only, or with both the VA and Rhizobium, produced more biomass than those inoculated with Rhizobium only ($P<0.05$) and non-inoculated ($P<0.01$), especially in the case of adding phosphates (Table 1).

Table 1. Effects of inoculation with G. epigaeus and Rhizobium on growth of sunn crotalaria

Treatment	Height (cm)	Leaves number	Dry wt. of tops (g)	Root colonization (%)	Spores per 100g of dry soil
G**	94.9a*	30.6a	1.08a	99	226
R+G	81.9b	27.4b	0.91b	100	234
P+G	68.2c	24.6c	0.68d	95	220
R	68.1c	23.3d	0.54e	0	0
P+R+G	67.6c	26.7b	0.74c	98	224
P	55.9d	19.8e	0.38f	0	0
CK	50.8e	18.4f	0.35f	0	0
P+R	49.5e	18.1f	0.32f	0	0

* Values not followed by the same letters are significantly different at the 5 % level of probability using LSR test;

** G-- Glomus epigaeus; R-- Rhizobium; P-- phosphate fertillizer 4g per pot.

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VAM REDUCE THE INHIBITORY EFFECTS OF COMBINED N ON N_2 FIXATION IN ALFALFA

By: W.J. Robertson and C.D. Boyle

Keywords--Glomus macrocarpum, NH_4Cl, Rhizobium meliloti, acetylene reduction

Introduction

For the past 3 years we have been investigating various aspects of the mycorrhizal associations of alfalfa (Medicago sativa). One facet that we are currently studying is their role in legume nitrogen nutrition. It is well known that VAM can indirectly produce increased rates of N_2 fixation. In addition there is also evidence that they can directly incorporate combined N, particularly NH_4^+ (Ames et al. 1983). The objective of the following experiment was to investigate if VAM by combined N uptake can also decrease its inhibitory effects on N_2 fixation in alfalfa.

Methods and Materials

Five-day old alfalfa seedlings (cv. Iroquois) were grown in pots containing vermiculite saturated with a modified Hoagland's solution (0 ppm N, 20 ppm P). Plants were inoculated with an onion root-soil mixture containing either G. macrocarpum or no VAM fungi, and were watered with 3 mL of a bacterial soil filtrate containing 10^6 cells/mL of Rhizobium meliloti (Balsac). The alfalfa was grown under a light:dark period of 16:8 hours with corresponding temperatures of 25° and 20°C. The plants were watered weekly and the vermiculite resaturated with the modified Hoagland's solution after 4 weeks.

On the seventh week, at which time VAM were well established, pots were randomly chosen, saturated with either 0, 1, 3, 5, 10 or 50 mM NH_4Cl or 10 mM NaCl and returned to the growth chamber. There were 5 mycorrhizal and 5 non-mycorrhizal pots at each salt concentration with 3 plants/pot. After 1 week, N_2ase activity (C_2H_2 reduction) of whole plants was measured using gas chromatography.

Plants were then harvested and dry weights of shoots and roots determined. Roots were also rated on the numbers of nodules present. A small quantity of roots from each plant was stained and examined for the presence of VAM. N and P concentrations of shoots and roots were determined by standard methods.

Results and Discussion

In the absence of additional N, there were no differences in N_2ase activity between mycorrhizal and control plants (Table 1). The addition of 1 mM NH_4Cl caused significant decreases in C_2H_2 reduction by the non-inoculated alfalfa. However, in inoculated plants 5 mM NH_4Cl was required to similarly inhibit N_2ase. The addition of 10 mM NaCl also reduced N_2ase activity in the absence of VAM, suggesting that this repression might be due to increased salinity and not specifically to combined nitrogen. There were no significant differences between mean dry weights of shoots (0.941 g, 0.975 g) and roots (0.599 g, 0.642 g) of mycorrhizal and control plants respectively. The N concentrations of mycorrhizal and control shoots although increasing with increasing NH_4Cl concentrations, were collectively the same ($\bar{x}$ = 2.39 and 2.41% respectively). The N concentrations of roots followed a similar pattern ($\bar{x}$ = 1.83 and 1.87% respectively). VAM were not observed in the roots of control plants and there were no obvious differences in the quantities of nodules present in any of the plants. In addition the mean P concentrations of shoots of both mycorrhizal treatments were similar (0.142 and 0.137% respectively), but mycorrhizal roots had a slightly higher ($p = 0.02$) mean P concentration than controls (0.127 vs. 0.107%). At the completion of the experiment, all plants were healthy showing no signs of nutritional deficiencies.

This experiment indicates that G. macrocarpum can reduce the inhibitory effects of combined N on N_2 fixation in alfalfa. We propose that the external hyphae removed combined N from the rhizosphere and thus prevented it from inhibiting N_2ase. The similar N concentration in both mycorrhizal treatments suggests that the combined N was not transferred to the plant but remained in the external hyphae. Longer incubation times in the presence of combined N could have perhaps resulted in the transfer of N to the plant. The similar dry weights of shoots and roots and identical P concentrations of mycorrhizal and control plants suggests that the observed effect was not attributable to increased photosynthesis or differing metabolic rates. We are continuing this investigation to determine if these differences in N_2ase activity also occur: 1) in soils, 2) with different N sources and times of application and 3) with other VAM and different strains of R. meliloti.

Table 1. Effect of NH_4Cl and NaCl on N_2ase Activity of Alfalfa ± G. macrocarpum[1]

mM NH_4Cl	Acetylene-reducing activity[2]	
	+ VAM	- VAM
0	3.74 a	3.77 a
1	3.67 a	3.01 b
3	3.65 a	2.49 bc
5	2.98 ab	2.39 bc
10	2.30 b	1.65 c
50	1.43 c	0.98 d
10 mM NaCl	4.02 a	2.31 bc

1. Means in each column followed by different letters are significantly different at $p<0.05$.

2. u moles C_2H_4/h/pot.

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ERICACEOUS AND ORCHIDACEOUS MYCORRHIZAE

AMMONIUM ASSIMILATION IN *PEZIZELLA ERICAE*

BY

B. J. St.John, S. E. Smith, F. A. Smith and D. J. D. Nicholas.

Key words--ericoid mycorrhizas, GS, GDH.

Introduction

Ericaceous plants usually grow on acid soils where soil nitrogen is present as ammonium at low concentrations. Mycorrhizal infection results in increased plant growth and higher N content of host plants (Stribley & Read 1974). *Pezizella ericae*, an ericoid mycorrhizal fungus can use NH_4^+ as a primary nitrogen source in axenic culture (Pearson & Read 1975). This ability is potentially important in the N nutrition of the host.

Utilisation of NH_4^+ involves consideration of: NH_4^+ uptake and consequent proton extrusion; activities of enzymes of NH_4^+ assimilation viz. Glutamine synthetase/glutamate synthase (GS/GOGAT) and Glutamate dehydrogenase (GDH).

Results of preliminary experiments on fungal growth, pH changes in the medium and the activity of these 2 enzyme systems are presented.

Methods

Pezizella ericae was grown in stationary liquid culture in 100 ml conical flasks containing 35 ml NH_4^+ medium pH 6.45 (Pearson & Read 1975), incubated at 22°C, and mycelial mats harvested at intervals.

GDH activity was measured by the oxidation of NAD(P)H at 340 nm (Bhandari & Nicholas 1981). GS activity was measured by the transferase method (Ahapiro & Stadtman 1970). Enzyme activities are expressed as µmoles substrate converted/min/g. fresh weight of fungal tissue. Ammonia concentrations were determined using an ORION NH_3 electrode.

RESULTS AND DISCUSSION

Increase in dry weight of *P.ericae* was associated with a fall in concentration of NH_4^+ and a decrease in pH of the external medium (Fig.1). Uptake and assimilation of ammonium results in proton extrusion from the cells, causing acidification of the medium.

When the external concentration of ammonium was high, the fungus grew using GDH to assimilate NH_4^+ (Fig.2). As the concentration of NH_4^+ fell to a threshhold level of approx. 9 mM, GS also became active. GS remained active at very low concentrations of ammonium. This enzyme is therefore likely to be of more importance than GDH in the assimilation of ammonium from the low NH_4^+ concentration in soils with ericaceous vegetation.

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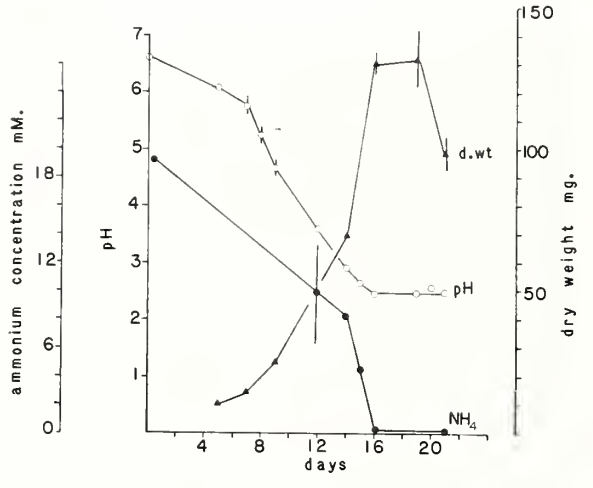

Fig.1 Changes in pH and NH_4^+ concentration in medium associated with growth (dry weight) of *P.ericae*.

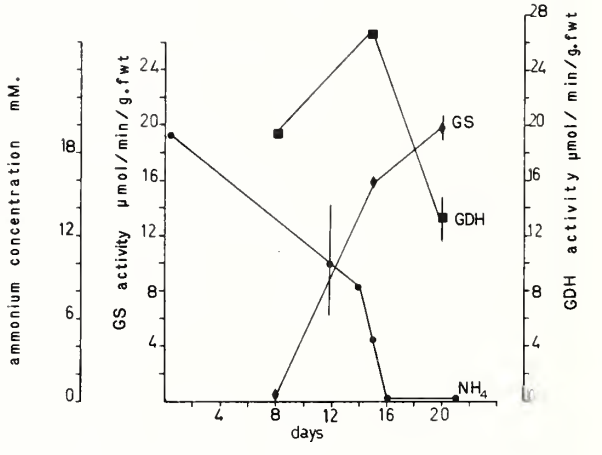

Fig.2 Changes in activity of GS and GDH and decrease in NH_4^+ concentration.

PHOSPHORUS UPTAKE BY MYCORRHIZAS OF THE ORCHID GOODYERA REPENS

Clare Alexander & Geoff Hadley
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Keywords - Translocation, inflow rate, ^{32}P phosphate

Introduction

Experiments were designed to measure P inflow rates to mycorrhizal and non-mycorrhizal orchid plants, and translocation of P by the endophyte, in order to compare the pattern of uptake and growth with that in other mycorrhizal systems.

Methods and Materials

Goodyera repens was grown from seed on potato dextrose agar or (for symbiotic material) on Pfeffer/cellulose agar together with the endophyte Rhizoctonia goodyerae-repentis. Plants that were 4-8 cm tall with up to 7 leaves were transplanted into perlite/vermiculite, with Melin Nilssons mineral solution, when used for experiments. The fungicide thiabendazole (TBZ) was used to suppress the mycorrhizal fungus as required.

Results and Discussion

To compare the uptake of phosphate in uninfected and mycorrhizal plants, they were set up in small pots and moistened with a P-free nutrient solution (Fig. 1). After two weeks the endophyte from the mycorrhizal plants had colonized the substrate and ^{32}P was added. Plants were harvested four weeks later and uptake of ^{32}P was assessed.

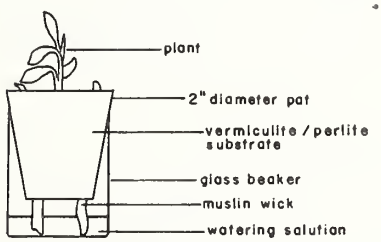

Figure 1. System for investigating P uptake.

Uptake by mycorrhizal plants was much greater than in the uninfected controls. (Table 1) and the difference was highly significant.

Table 1. Uptake of ^{32}P by mycorrhizal and uninfected plants (n = 8).

	Uninfected (control)		Mycorrhizal
Amount of ^{32}P in plant (dpm)	63,154	**	1,223,350
Amount of ^{32}P in shoot (dpm)	1,610	***	270,240
Uptake per mm rooting length (dpm mm^{-1} wk^{-1})	151	***	11,642
Inflow rate of P mole/cm root/s x 10^{-18}	0.44	***	76.88

Uptake of phosphate under two P regimes was examined using mycorrhizal plants from the field, put in small pots and maintained under low (10 µg P ml^{-1}) or high (1000 µg P ml^{-1}) P regimes. Half the plants in each regime were treated by including 10 µg ml^{-1} of TBZ to produce "non-mycorrhizal" plants with no external mycelium, but otherwise identical to mycorrhizal ones. After 8 weeks ^{32}P was added. Plants were harvested after 12 weeks and growth and P inflow rate calculated.

Relative growth rate was significantly ($P < 0.05$) reduced in plants treated with fungicide under the low P regime (Fig. 2). Also, in this treatment (but not others) there was a significant correlation between rooting length and ^{32}P uptake.

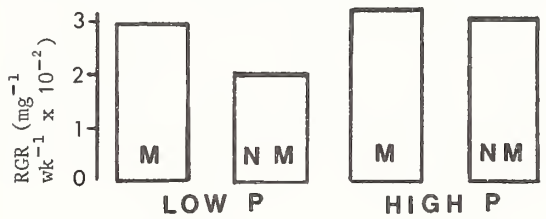

Figure 2. Relative growth rate of mycorrhizal (M) and non-mycorrhizal (NM) plants under low and high phosphate regimes.

The P inflow rate of mycorrhizal plants under the low P regime was three times that of plants treated with fungicide (Table 2). Under the high P regime, inflow rates were much greater, this presumably being "luxury" uptake with no associated increase in growth rate.

Table 2. Mean inflow rate of P (mole/cm rooting length s^{-1} x 10^{-15}) to mycorrhizal and "non-mycorrhizal" (fungicide-treated) plants.

	Mycorrhizal		TBZ-treated
Low P (10 µg P ml^{-1})	3.09	**	0.77
High P (1000 µg P ml^{-1})	163.0	*	102.0

* $p < 0.05$ ** $p < 0.001$

Uptake of phosphate over defined distances was examined in mycorrhizal plants, some of which were treated with TBZ, in petri dishes fitted with a root barrier. After 10 weeks the endophyte from non-fungicide treated plants had colonized the substrate. ^{32}P was injected 3, 6 or 9 cm from the root barrier and plants were harvested two weeks later.

^{32}P appeared in the root systems of five out of six mycorrhizal plants at each distance and was clearly translocated through mycelium up to 9 cm. One of six fungicide treated plants contained ^{32}P supplied at a distance of 3 cm, but some mycelium was present in this case.

Conclusions

Mycorrhizal infection of G. repens increases the efficiency of P uptake and under conditions of P stress this leads to a significant enhancement of growth. The results suggest that this orchid system is remarkably similar to other mycorrhizas in its ability to enhance the phosphate nutrition of the host plant.

CARBON NUTRITION OF ORCHID PROTOCORMS AND PLANTS

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Keywords - Goodyera repens, Rhizoctonia, translocation

Introduction

The importance of mycorrhizal infection in providing carbon for the development of orchid protocorms has been well established. It has been hypothesised that the need for an exogenous carbohydrate source is outgrown with production of the first leaf but there is no direct evidence from past work. We tested this hypothesis by studying movement of C from an insoluble ^{14}C source into protocorms and plants.

Methods and Materials

Goodyera repens was grown from seed on Pfeffer dextrose agar, or on Pfeffer cellulose agar together with its endophyte Rhizoctonia goodyerae-repentis. Plant material for experiments was PROTOCORMS - non-green, undifferentiated seedlings; PLANTLETS - photosynthetic individuals, up to 50 mg f. wt. with one or two roots and up to four leaves; or PLANTS - larger photosynthetic individuals with up to seven leaves.

The systemic fungicide thiabendazole (TBZ) was used at 1 μg ml^{-1} in agar to inhibit the endophytic fungus and provide 'non-mycorrhizal' plant material.

Insoluble ^{14}C material was used in the form of plant residues, dialyzed to remove soluble substances, and applied in suspension.

Results and Discussion

Uptake of ^{14}C into PROTOCORMS was examined using the system shown in Fig. 1, with four mycorrhizal protocorms in each well. The endophyte grew out to colonize the large petri dish but hyphal connections from well no. 2 were prevented by turning it frequently. Protocorms in this well therefore monitored any $^{14}CO_2$ fixation. ^{14}C was put into the channel and after 14 days was assayed in individual protocorms.

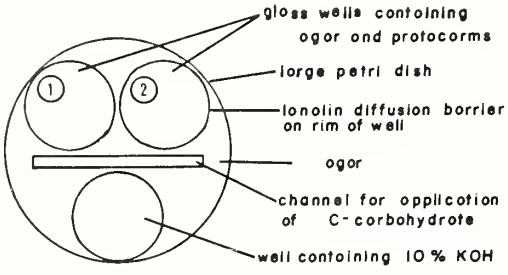

Figure 1. Dish system for ^{14}C translocation study.

Low levels (max. 150 dpm) of radioactivity were found in control protocorms. Translocation occurred in three dishes (mean count of 3100 dpm) while the fourth dish developed only sparse mycelium and no counts were recorded.

Translocation of ^{14}C into PLANTLETS was investigated using a similar system to that in Fig. 1, but with four smaller (1 cm diam.) wells, two of which contained 0.1 μg/l of TBZ in the agar. One plantlet was placed in each well and when the endophytic fungus had grown from the (non-fungicide) well to colonize the main dish, ^{14}C was applied.

After two weeks, all plantlets contained label and in both the treatments the amount of ^{14}C was correlated with plantlet weight (Fig. 2). However, the plantlets with external mycelium contained significantly more ^{14}C than those without (i.e. fungicide treated ones) and the correlation between uptake and weight was weaker. This suggested the involvement of some factor other than photosynthetic area, probably the mycorrhizal mycelium, in the uptake of ^{14}C.

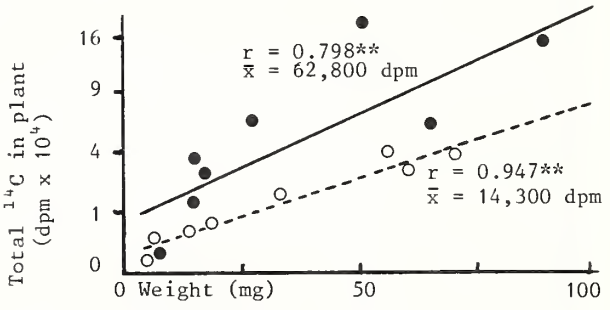

Figure 2. ^{14}C uptake in relation to weight in plantlets with (•) and without (o) external hyphae.

Uptake of ^{14}C into PLANTS was examined by isolating the root system (Fig. 3), thus avoiding photosynthetic $^{14}CO_2$ fixation. Two batches of mycorrhizal plants were set up, one a control with 1 μg ml^{-1} of TBZ to inhibit the endophyte. In this case the fungus was separately inoculated to the main chamber and colonized the ^{14}C source, thus producing $^{14}CO_2$ as in the mycorrhizal chambers, but was not in contact with the plant.

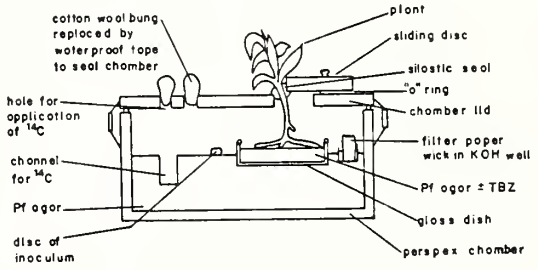

Figure 3. Chambers used to isolate root system.

^{14}C was assayed in root and shoot systems two weeks after application of the ^{14}C source. Negligible counts (< 1000 dpm) were present in all of the plants and there was no difference between the treatments. Clearly, translocation from the C-source was not taking place.

The results showing translocation into protocorms of G. repens extend earlier findings using ^{14}C glucose. Movement into plantlets is also demonstrated; this would be advantageous to young plantlets in the early stages of development in the field. In contrast, mycorrhizal plants do not take up carbon via their endophytic fungus.

ULTRASTRUCTURE OF PYROLA MYCORRHIZAE

By

Diane C. Robertson & Jack A. Robertson

Keywords--arbutoid, Ericales

Introduction

The genus Pyrola (Ericales) characteristically has an ectendomycorrhizal association which has been termed an "arbutoid" mycorrhiza. Only one detailed ultrastructural study of an arbutoid mycorrhiza has been published (Fusconi & Bonfante-Fasolo, 1984). This paper presents the results of an ultrastructural analysis of mycorrhizae from six species of Pyrola.

Methods and Materials

From July 3 to Aug 8, 1979, numerous collections of Pyrola spp. were made from a variety of sites in northwestern Montana. Species examined were: P. asarifolia, P. chlorantha, P. minor, P. picta, P. secunda and P. uniflora.

Mycorrhizal roots from each species were prepared for electron microscopy as described previously (Robertson & Robertson, 1982).

Results and Discussion

The hyphae on the root surface varied from a loose weft to an abundant mass with numerous strands, but no organized sheath was observed. Infection began with the formation of a Hartig net several millimeters behind the root tip. Hyphae from this net subsequently grew into each epidermal cell, forming masses of intracellular hyphae (Fig. 1). These hyphae were surrounded by the host plasmalemma and a matrix material, presumably of host origin. During the stage of mature infection the host cytoplasm was dense-staining and filled with organelles (Fig. 2). The host vacuoles often had tannin-like deposits along their tonoplasts.

Senescence of the symbiosis began with the gradual degeneration of the host cytoplasm, whereby it became dark and vesiculated with loss of its organelles (Fig. 3). The fungal hyphae and matrix material appeared essentially unchanged at this stage, but eventually degenerated and collapsed.

The fungal partners were normally basidiomycetes with dolipore septa, but one ascomycetous infection (distinguished by simple septa and Woronin bodies) was examined and found to have a similar mycorrhizal organization. It differed in having an intermittent Hartig net.

These results are similar to those reported for Arbutus (Fusconi & Bonfante-Fasolo, 1984) except for the absence of a sheath in Pyrola. This was consistent in spite of the variety of sites from which collections were made.

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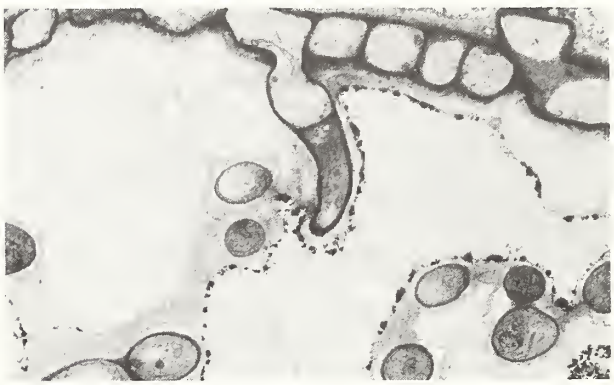

Figure 1. Pyrola secunda--early intracellular infection from Hartig net. X3,920

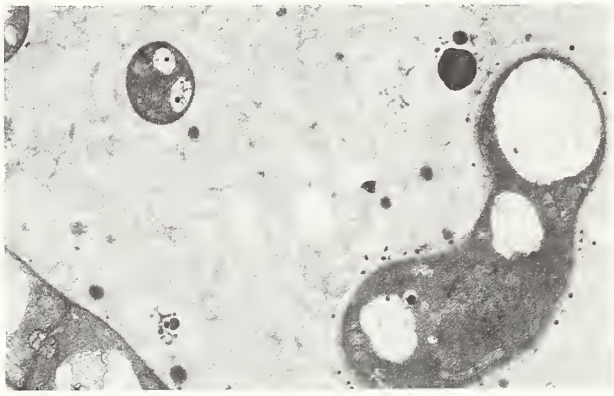

Figure 2. P. chlorantha--mature intracellular infection. X6,160

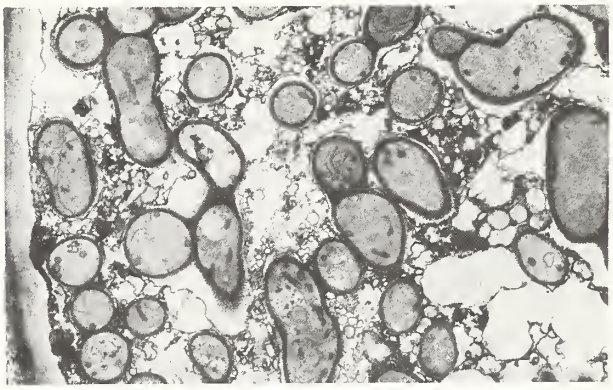

Figure 3. P. secunda--early senescence with degenerated host cytoplasm. X3,920

This work was completed at The Ohio State Univ., Dept. of Plant Pathology and the Electron Microscopy Laboratory, Ohio Agricultural Research & Development Center, Wooster 44691

PROTEINS AS NITROGEN SOURCES FOR HYMENOSCYPHUS (=PEZIZELLA) ERICAE

S. Spinner and K. Haselwandter

Keywords--gliadine, bovine serum albumine, ericoid mycorrhizal fungus

Introduction

Using ^{15}N-labelled NH_4^+ and glutamine Stribley and Read (1975) have shown that nitrogen uptake into plants is largely facilitated by the fungal symbiont. There is evidence that the ericaceous endophyte utilizes inorganic nitrogen and simple organic nitrogen compounds (Pearson and Read 1975), but not humic or fulvic acids (Stribley and Read 1980). This leaves the question whether the fungal symbiont of ericaceous plants is capable of using less complex organic soil constituents like proteins as nitrogen sources. In order to investigate this H. ericae was grown in liquid culture with different nitrogen sources, its growth rate determined, and total nitrogen content and pH of the culture medium were measured at regular intervals.

Material and Methods

Fruitbodies of Hymenoscyphus ericae (Read) Korf et Kernan ap. Kernan and Finocchio (1983) were obtained from Dr D.J.Read, England. Mycelial cultures were established by germination of ascospores on water agar (0,5 % agar) under aseptic conditions. Mycelial discs (6 mm Ø) from such cultures were used to inoculate different growth media:

1. Basal medium (BM) of Pearson and Read (1975) with 0,2 % $NH_4H_2PO_4$ (= 17,3 mM N) as nitrogen source.
2. Bovine serum albumine medium (BSA): Same composition as 1, but $NH_4H_2PO_4$ substituted with equimolar concentration of BSA (= 0,164 %). BSA was added as sterile-filtered aqueous solution after autoclaving the rest of the medium.
3. Gliadine medium (GL): Same composition as 1, with equimolar nitrogen concentration of gliadine (= 0,146 %) instead of $NH_4H_2PO_4$. Gliadine was added as sterile-filtered ethanolic solution.

The cultures were incubated at 18°C and shaken at 150 rpm. At 3 days intervals the mycelium was harvested and its dry weight determined. Total nitrogen content and pH of the culture medium were measured.

Results and Discussion

As shown in Fig. 1 H. ericae is capable of growing on media with proteins as sole nitrogen source. The lag-phase on BM, however, is shorter than on BSA or GL. BSA is utilized more quickly than gliadine. This might be due to the greater solubility of BSA in water. Changes in pH of the culture medium are more pronounced in the BM than in those with the organic nitrogen sources (Fig. 2), probably because the proteins have some buffering capacity.

Comparison of Fig. 1 and Fig. 2 shows that the increase in biomass is accompanied by a decrease of total nitrogen content of the culture medium. Greatest reduction of total nitrogen content coincides with the largest increment of fungal biomass. It is interesting to note that after 18 days yield of mycelium is higher in BM than in BSA where it exceeds that in GL what is reflected in the shift of total nitrogen content of the medium.

Many fungi including ascomycetes produce proteases during a non-autolytic phase of growth (Cohen 1980). The results indicate that H.ericae is also capable of utilizing proteins as nitrogen sources. This is of particular interest as so far it has been shown that the fungal symbiont of ericaceous plants utilizes NO_3^-, NO_2^-, NH_4^+ and amino acids as nitrogen sources. The production of proteolytic enzymes by the fungal endophyte might enable ericaceous host plants to obtain access to nitrogen sources which would otherwise be unavailable.

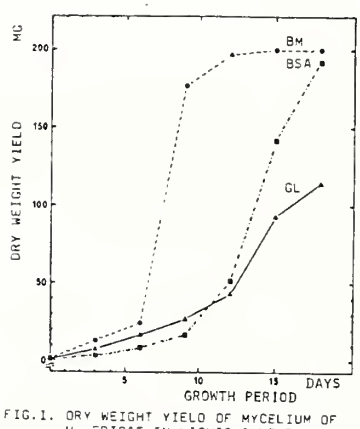

FIG.1. DRY WEIGHT YIELD OF MYCELIUM OF H. ERICAE IN LIQUID CULTURE WITH DIFFERENT NITROGEN SOURCES.

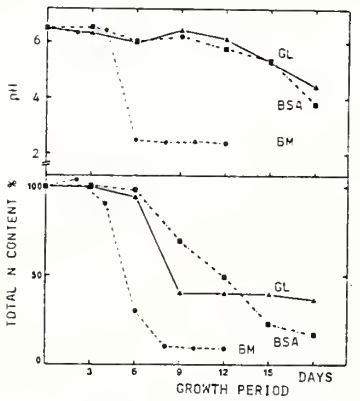

FIG.2. CHANGES IN pH AND TOTAL NITROGEN CONTENT OF CULTURE MEDIUM WITH TIME.

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ORCHIDACEOUS AND PHYCOMYCETE COENDOPHYTES: PEYRONEL REVISITED

By

P.G. Williams and M.J. Milligan

Keywords--Rhizoctonia, vesicular-arbuscular mycorrhiza, double infection, historical, plant growth effects.

Introduction

Nowadays it is generally held that the roots of most plants are colonised more or less intimately and with variable regularity by diverse, comparatively harmless fungi. However, in the early days of research on endomycorrhiza many workers believed that the aseptate type of endophyte which formed arbuscules and vesicles was regularly associated with a particular septate endophyte. Debate about the so-called "double infection" subsided after the late 1920's and has never been resumed. This poster summarises findings which indicate that interest in certain septate fungi closely associated with V-A mycorrhizal endophytes ought to be revived.

Early observations

Before the current period of experimentation, knowledge about endomycorrhiza was obtained by describing natural infections. According to such descriptions (see Peyronel 1923a), following the colonisation of roots by the aseptate type of endophyte, an infection by a septate endophyte occurred which was superimposed on the first. The septate endophyte formed intracellular coils and became intimately mingled with the aseptate endophyte. The two fungi sometimes occurred together in the same cell. Benjamino Peyronel (1923b,1924) demonstrated that coinfection of roots by aseptate and septate endophytes occurred in a wide variety of flowering plants. He also challenged the view of several writers that the two fungi belonged to a single group: although he was unable to isolate the aseptate endophyte, which he believed was a Phycomycete, he succeeded in isolating the septate fungus from the roots of a dozen different plants including wheat, potato, carrot, tobacco, vine, etc. All his isolates were similar. They were sterile, septate fungi which, in older cultures, formed monilioid hyphae and clumps of swollen cells and sporodochia. These features, together with the appearance of colonies on agar, suggested to Peyronel that the fungi were similar to Rhizoctonia repens and R. languinosa, two of the orchid mycorrhizal fungi described by Bernard in 1909.

Present observations

Research in this laboratory has identified a group of mainly undescribed Rhizoctonias (sensu lato) whose intimate association with V-A endophytes and orchidaceous nature recapitulates the phenomena which preoccupied Peyronel and others during the first decades of this century. The following is a dossier of presently known attributes of the Rhizoctonias studied in this laboratory since 1977.

1. Occurrence: as spore-like cells inside internal (Fig. 1a) and external vesicles of Glomus spp; also as early outgrowth hyphae from washed roots of pot culture plants and field plants incubated on agar (Fig. 1b).
2. Characteristics: narrow (2-4 um), irregularly septate, multinucleate hyphae producing ± spherical chlamydospores, monilioid chains, sporodochia; pale, sebacious colonies; dolipore septa; intra- and inter-cellular mycelia formed in root cortex of non-orchid plants.
3. Identity: all pot culture isolates are previously undescribed Rhizoctonias; three field isolates are similar to R. globularis Saksena & Vaartaja; J.H. Warcup reports that the teleomorph of one pot culture isolate and two field isolates (with R. globularis anamorph) is similar, but not identical, to Sebacina vermifera Oberwinkler (Tremellales, Basidiomycotina), a common endophyte of certain Australian terrestrial orchids (Warcup 1981). All of 10 isolates tested caused symbiotic germination of seed of Microtis spp.(Orchidaceae).
4. Biological activity: inoculating non-orchid host plants with Rhizoctonia mycelia has effects only when V-A mycorrhizal fungi are present:-

in incompletely disinfested soil: infection, or colonisation (or both) by residual V-A endophyte is enhanced in inoculated plants;

in natural soil: growth of inoculated plants may be increased, decreased or unaffected, depending on soil, host plant and strain of Rhizoctonia.

Conclusion

The orchidaceous Rhizoctonias associated with V-A mycorrhizal fungi deserve renewed investigation.

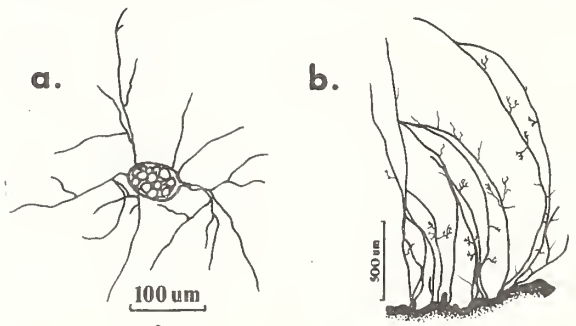

Fig. 1. Occurrence of Rhizoctonias, (a) as spore-like cells inside Glomus vesicles and (b) as outgrowth hyphae from pot culture root.

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TAXONOMY, CLASSIFICATION AND CHARACTERIZATION

SPORULATION OF ENDOGONE PISIFORMIS IN PURE CULTURE

By

Shannon M. Berch and Michael A. Castellano

Keywords--Endogone pisiformis, axenic culture, axenic sporulation

Introduction

Endogone pisiformis Lk. is one of the few Endogonaceae that has been grown and maintained through repeated transfers in axenic culture on artificial media (Berch and Fortin 1983a). Under monoxenic and nonsterile conditions this fungus did not form ectomycorrhizae with Pinus sylvestris L. or vesicular-arbuscular mycorrhizae (VAM) with Allium porrum L. Sporulation did not occur under the growth conditions tested.

To examine the comportment of this fungus with other potential host plants under different growth conditions, we used the pure culture synthesis method described by Molina and Palmer (1982). The fungus flourished under these conditions and, to our surprise and delight, produced typical zygosporocarps.

We report here preliminary results of a procedure to define nutritional requirements and cultural conditions conducive to sporulation of E. pisiformis.

Materials and Methods

An E. pisiformis isolate from the Quebec City, Canada region was transferred from modified Melin-Norkrans (MMN) agar medium to liquid MMN. Ten ml of liquid culture was used to inoculate Douglas fir, western hemlock, lodgepole pine and chives (Allium schoenoprasum L.). Large glass tubes containing seedlings and fungus with peat, vermiculite, and MMN nutrients were maintained in a water bath under flourescent and incandescent illumination (Molina and Palmer 1982). Also, water-washed mycelial inoculum was used in a factorial experiment to determine the nutritional and cultural requirements for fungal growth and sporulation.

Results

Endogone pisiformis did not grow in the absence of either simple sugar or nutrients. Limited mycelial growth occured initially in the treatment which lacked sugar and a plant, but soon stopped. Presence of a plant accelerated but was not requisite for sporulation. Sporulation occured after three months in all treatments containing plants and in the "complete minus plant" treatment inoculated with unwashed mycelium. Washing the mycelium slowed the growth of E. pisiformis but preliminary observations suggest that washed mycelium may recover.

Zygosporocarps were formed at the walls of the glass tubes near water-line where a temperature differential of approximately 5 °C exists.

Discussion

Results to date indicate that E. pisiformis requires either a plant or simple sugar to grow and is incapable of using peat as a sole source of carbon. Endogone pisiformis may have little or no ability to decompose complex organic compounds and a typically zygomycetous dependency on simple sugars or a living host. Washing the mycelium retarded growth of the fungus, but it is possible that less strenuous washing may remove the nutrient solution without being detrimental. Ectomycorrhizae were not formed on the conifers tested, but the root surface was colonized by mycelium. Chives did not form VAM, but senescent roots were invaded by vesicular hyphae.

In nature, E. pisiformis sporulates epigeously on various substrates where temperature, humidity, and light differentials certainly occur. In our experimental procedure, little difference in light intensity above and below the water-line would occur since the culture tubes are transparent. Similarly, humidity within the closed tubes would probably not vary markedly. However, a temperature differential of 5°C existed in the tubes at water-line and we hypothesize that this directly influenced fungal sporulation.

It would be interesting to examine the role of temperature differential in stimulating sporulation of E. pisiformis on simple, artificial media. If conditions conducive to sporulation can be defined, then it will become possible to study the life cycle of E. pisiformis in detail since its zygospores readily germinate on agar media and produce viable mycelia (Berch and Fortin 1983b).

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A SPECIES OF ACAULOSPORA THAT FORMS SPOROCARPS AND TWO SPECIES OF ENDOGONE THAT DO NOT

by

Shannon M. Berch

Key Words--Endogonaceae, taxonomy, sporocarp, Acaulospora, Endogone

Introduction

One of the traditional reasons for including taxa in the Endogonaceae was the formation of sporocarps (Thaxter 1922). As the number of studied species has increased, it has become apparent that sporocarp formation is a variable characteristic. However, until recently no sporocarpic Acaulospora species or nonsporocarpic species of Endogone have been formally described.

Of the vesciular-arbuscular mycorrhizal fungi, in the past only Glomus and Sclerocystis have been known to form sporocarps. While examining unidentified herbarium material collected separately from Arizona and Pakistan, I encountered sporocarps of an undescribed species with Acaulospora-like spores. This species is informally described here as Acaulospora sporocarpa nom. ined.

Warcup (1975) discussed nonsporocarpic Endogone species, but the majority of Endogone species seem to be only sporocarpic. I have examined collections of two species of Endogone from Oregon that either do not form sporocarps or form sporocarps of a few spores only. Endogone incohaerens nom. ined. and E. alveolata nom. ined. are informally descibed here.

Taxonomic part

1. "Acaulospora sporocarpa" nom. ined.

Sporocarps of "Acaulospora sporocarpa" from Pakistan can attain 2 cm and consist of hyphae, spores, vesicular swellings, and soil particles. Dark brown to black, ellipsoid to subglobose spores form laterally on sporogenous hyphae subtending terminal hyphal swellings. Spores may bear a prominent attached hypha at maturity and, in the absence of the terminal hyphal swelling, may superficially resemble spores of Glomus. When broken, the pigmented outer spore wall can be seen to enclose the hyaline, somewhat membraneous inner wall.

In addition to the spores and terminal, hyphal swellings involved in spore formation, sporocarps of A. sporocarpa contain intercalary or terminal hyphal swellings reminiscent of those of A. gerdemannii Schenck & Nicolson and of vesicles or thin-walled spores of Glomus species.

2. "Endogone alveolata" nom. ined.

Single, isolated zygospores of "Endogone alveolata" are enclosed in mantles of hyphae. Zygosporangia have an alveolate reticulum and enclose a hyaline zygospore. Gametangia are unequal and pigmented. Young mantles form around immature, hyaline zygospores. The mantle surrounding the zygosporangium bears fine hyphae projecting from vesicular swellings. The pigmented zygosporangium of E. alveolata is pitted.

3. "Endogone incohaerens" nom. ined.

Red-brown zygospores of "Endogone incohaerens" are enclosed in a mantle of hyphae and subtended by two unequal gametangia. Interwoven, branched hyphae comprise the zygospore mantle. The pigmented zygosporangium wall encloses an inner hyaline zygospore wall.

Conclusions

In some species of Glomus, sporocarp formation is a facultative characteristic and therefore of limited taxonomic importance. Similarly, and since other characteristics correspond, there is no reason to exclude "Acaulospora sporocarpa" from the otherwise nonsporocarpic Acaulospora species.

Based on a number of characteristics, it may be necessary to separate E. incohaerens nom. ined. and E. alveolata nom. ined. as well as some other species from Endogone. Detailed study of other Endogone species and their biology will be done before making a major taxonomic change.

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A NEW STAINING PROCEDURE FOR VAM

By

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Keywords--vesicular-arbuscular mycorrhizae, chlorazol black E, staining procedure, morphology.

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Introduction

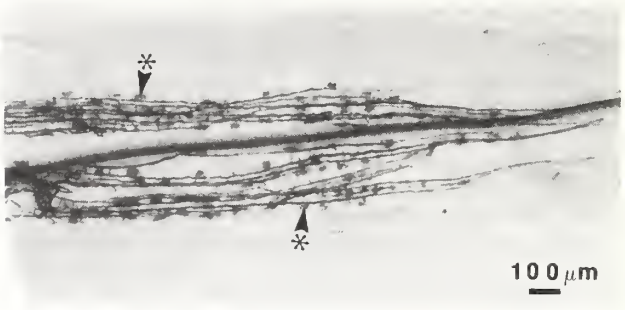
A new staining procedure using chlorazol black E is presented for staining vesicular-arbuscular mycorrhizal (VAM) fungi in cleared roots. In a comparative study, chlorazol black E is superior to previously used stains (acid fuchsin, trypan blue, aniline blue) for showing details of internal hyphae and particularly arbuscules. This clearing and staining procedure, combined with Nomarski interference contrast microscopy, reveals details of arbuscule structure not evident with previous techniques. The procedure should allow for more accurate assessments of roots for degree of colonization by vesicular-arbuscular fungi and may be useful in the taxonomy of this group of fungi.

Preparation of Endomycorrhizal Roots for Light Microscopy

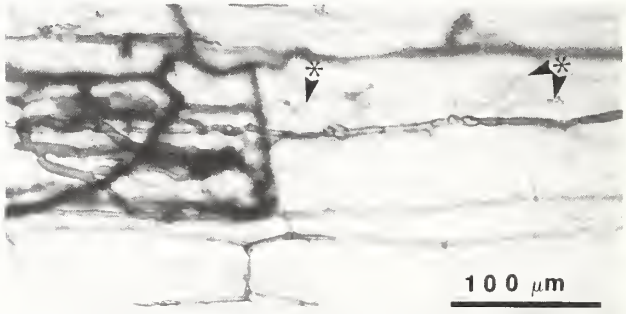
The dye chlorazol black E is introduced as a substitute for acid fuchsin or trypan blue in the VAM clearing and staining techniques outlined below:

1. Wash roots thoroughly and fix overnight, or store in formalin-acetic acid-alcohol (FAA).
2. Rinse with several changes of tap water to remove FAA.
3. Transfer samples into a 10% (w/v) potassium hydroxide solution in autoclave-resistant jars. Clear in autoclave for 15 minutes at 121°C. Samples with delicate roots may require shorter clearing times.
4. After samples have cooled, rinse with several changes of tap water followed by deionized water.
5. Transfer roots into equal volumes of 85% lactic acid, glycerin, and distilled water with 0.1% (w/v) chlorazol black E for 1 hour or longer at approximately 90°C. Prepare the staining solution several hours before use and allow undissolved stain particles to settle out. Samples previously stained with acid fuchsin and stored in glycerin may be restained with chlorazol black E.
6. Transfer stained roots into glycerin for storage or observation.
7. After destaining overnight in glycerin, mount roots on slides using Hoyer's mounting fluid with the chloral hydrate reduced to 2%.

Morphology of mycorrhizal leek roots obtained from growth room-grown plants, stained with chlorazol black E.

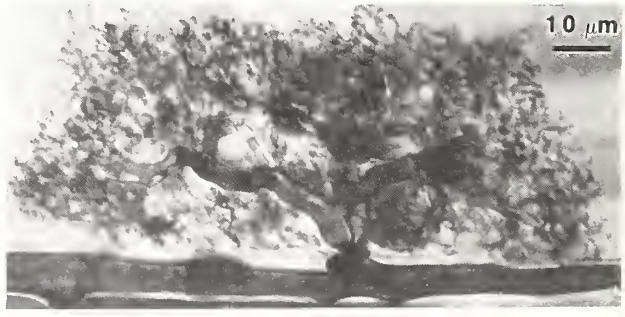


A young Glomus mosseae infection unit with many arbuscules (*)



A very young infection of G. monosporum with several developing arbuscules (*)

Three young G. mosseae arbuscules showing trunk and some branch hyphae.



An older (G. mosseae) arbuscule with numerous fine branch hyphae.

Reference

Brundrett, M.C., Y. Piché and R.L. Peterson. In press. A new method for observing the morphology of vesicular-arbuscular mycorrhizae. Can. J. Bot.

WALL STRUCTURE IN THE SPORES OF GLOMUS SPECIES PLURIMAE.

By
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Key words:cell wall,Glomus,taxonomy,
ultrastructure,VAM fungi.

Introduction

Recent papers on VAM fungi taxonomy indicate the sporal wall as a feature of important diagnostic value in identifying a species within a genus (Trappe and Schenck, 1982;Berch and Fortin,1983).
However,some problems arise from this statement,because the structure of the wall is not always well evident in light microscopy(LM)and-according to Walker(1983)-the terminology used in wall description has not been consistent,resulting in confusion during identification.
The aim of this work was to verify:1)if there is an agreement between LM descriptions and ultrastructural observations;2) which is the fine architecture of the walls described as laminated,'unit',etc..3)if the wall has actually a diagnostic value.

Materials and Methods

Chlamydospores of Glomus epigaeum,G.macrocarpum,G.caledonium,G.clarum,G.constrictum,G.occultum,G.mosseae and G.fasciculatum'E_3' were partly observed by LM and partly fixed and prepared for electron microscope observations.

Results

As observed in LM the wall of G.epigaeum is usually described as made up by two layers.Ultrastructural observations show that the outer wall has a homogeneous texture, consisting of ordered parallel fibrils, whereas the inner wall is regularly organized with an arched fibrillar texture,typical of insect chitinous cuticles.The two walls are separated by a dark line,whose main component is sporopollenin.Soil bacteria can lyse the outer wall,but they were never seen to cross the dark line.The same arched fibrillar structure is observed in the larger part of the wall of G.macrocarpum,whilst the outer zone,described as an ephemeral layer by LM,is not well defined and seems to be made up by fibrillar units sloughing into the soil.
A similar pattern is observed in G.caledonium,where the larger part of the wall is formed by ordered and stacked fibrils,that -at the interface with the soil-are sloughing off.Two walls are clearly seen in G. occultum,G.constrictum and G.clarum.In the latter,the outer wall shows an amorphous texture and is colonized by soil bacteria: the inner wall has a completely homogeneous structure,being formed by stacked ordered fibrils without lamellations.A homogeneous texture is also present in the wall of G.mosseae ,the external part of which does not show signs of sloughing off.G.fasciculatum 'E_3'spore walls show variable features ,as some of them appear to be homogeneous with a fibrillar structure,whereas others reveal separated layers with a plywood architecture.

Conclusions

Results obtained with the species examined show a general agreement between LM and ultrastructural observations,even if some important differences exist,mostly regarding the outer wall in G.caledonium and G. macrocarpum.Ultrastructural analysis allows to understand the fine architecture of the wall components ,giving a firm meaning to the terminology used till now.
Fibrillar units embedded into an amorphous matrix can show a continuous ordered deposition,giving rise to'unit'walls.Alternatively,the fibril deposition causes an arc texture,due to layers in which regularly ordered fibrils are rotated through a small angle with respect to the previous layers(Bonfante-Fasolo and Vian,1984).
This type of organization is responsible of the'laminated' walls.
In conclusion,it is confirmed that the wall is actually a trustable taxonomic criterion for identifying VAM spores,as all the samples examined in the present study show a different wall architecture, suggesting a different rhythm of fibril deposition during spore morphogenesis.

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AUTOFLUORESCENCE PHENOMENON IN VAM FUNGI.

By
Y. Dalpé

Keywords: Gigaspora calospora, Glomus clarum G. mosseae, G. macrocarpum, G. epigaeum, Sclerocystis rubiformis, UV fluorescence.

Introduction

The UV fluorescence characteristics of fungi, especially of vesicular arbuscular mycorrhizal fungi have not been explored sufficiently. Autofluorescence response to a blue excitation light (455-490 nm) has recently been used to detect intraradical arbuscules in several hosts (AMES & al, 1982).

Preliminary studies indicated that other structures formed by VAM fungi are autofluorescent under different wavelengths.

Methods and Materials

Spores of Gigaspora calospora, Glomus clarum, G. mosseae, G. macrocarpum, G. epigaeum, G. sp and Sclerocystis rubiformis were isolated from the soil by sieving and filtration. Colonized roots of Tagetes erecta (Marigold) were obtained from pot cultures inoculated by the same spores in a growth chamber.

Azygospores and chlamydospores were untreated before mounting in water however roots were cleared in 10% KOH at 90°C for 10 to 30 minutes Untreated colonized roots gave identical result but were more difficult to observe.

Differential Interference Contrast microscopy, DIC, was obtained with a Nomarski prism coupled to CF Plan Achromat objectives 20X and 40X (Optiphot, Nikon). UV fluorescence was induced utilizing an episcopic fluorescence attachment "EF" on an Optiphot stand, equipped with a high pressure mercury lamp set, exciter filters of 330-380 nm, absorption filter of 420K and CF UV-F objectives of 10X and 40X.

Results and Discussion

A dark pigmentation in the spore walls inhibited UV fluorescence. In lightly pigmented cells, components of the thick cell wall, of the granular cytoplasmic content and of the subtending hyphae fluoresce. The autofluorescence of the lipidic droplets inside the spores seems to fade with the aging process.

In all the cases studied, both intra and extraradical vesicles and hyphae have a bright pale blue autofluorescence. The advance of hyphae through a root can easily be followed. The arbuscules which fluoresce under blue light (AMES & al, 1982) do not fluoresce under UV.

The difference in the wavelength excitation fluorescence between arbuscules and other structures as intra and extraradical spores, vesicles and hyphae reinforces the hypothesis of a specialized status attributed to arbuscules in the nutrition of VAM fungi.

The use of adapted fluorescence technique in the study of VAM fungi presents definite advantages
- in following the growth and maturation of the living organism,
- in rapidly estimating their level of colonisation

It remains the best non destructive method of microscopy which requires no special staining procedure and no time delay for the observation of the specimens

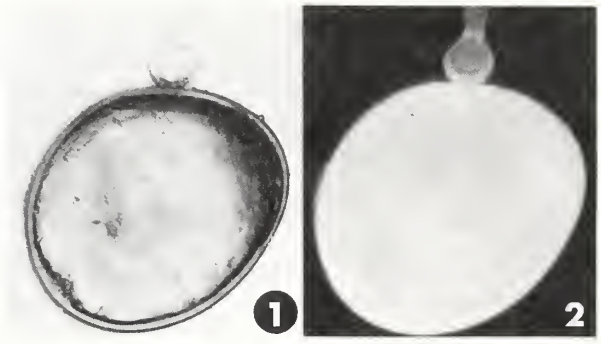

Fig. 1-2, Azygospore of Gigaspora calospora X206; Fig.1, with DIC; Fig.2, under UV fluorescence.

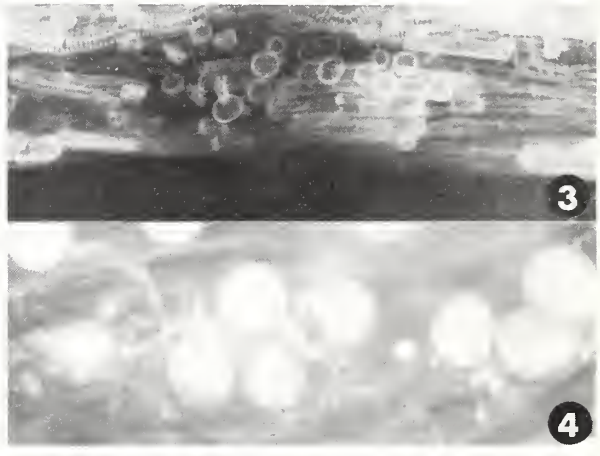

Fig.3-4, Autofluorescence under UV light of vesicles and hyphae inside roots of Tagetes erecta. Fig.3, Glomus mosseae X103; Fig.4, Glomus epigaeum X206.

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ULTRASTRUCTURAL EVIDENCE FOR THE ASCOMYCETE-LIKE NATURE OF GLAZIELLA AURANTIACA

By

J.L. Gibson, J.W. Kimbrough and G.L. Benny

Keywords--Endogonaceae, septum, unispored ascus, spore ontogeny, cytology, taxonomy

Introduction

Glaziella aurantiaca (Berk. & Curt.) Cooke is a sporocarpic fungus of tropical lowlands that has traditionally been included in the Endogonaceae because the spores were assumed to be chlamydospores (fig. 1). Thaxter (1922) included Glaziella in the Endogonaceae, although somewhat hesitantly because of the regular septation of the sporocarpic hyphae. Gerdemann and Trappe (1974) include the monotypic genus in their monograph and state that the mycorrhizal status is unknown. Gibson (1984) found the septa to be typically ascomycetous and, on this basis, transferred Glaziella to the Fungi Imperfecti, due to lack of knowledge regarding its teleomorphic stage.

The purpose of this study is to examine the sporocarpic tissues with light and electron microscopy in order to gain more information about the morphology, cytology, and teleomorphic nature of the fungus.

Methods and Materials

Since fresh specimens of Glaziella were not available for study, dried herbarium material, now deposited in the Tulane University Herbarium, New Orleans, Louisiana, U.S.A., was examined.

Small pieces of the rehydrated sporocarp wall were: 1) sectioned on a Cryostat freezing microtome, stained with lacto-phenol cotton blue and examined with a light microscope equipped with Normarski differential-interference contrast optics. 2) fixed in glutaraldehyde, dehydrated, embedded, and sectioned for observation with transmission electron microscopy (TEM). For scanning electron microscopy (SEM), razor blade sections of the dried sporocarp wall were coated with gold and observed directly.

Results and Discussion

The evidence from electron microscopy indicates that Glaziella is an ascomycete. In SEM the large spores are seen to lie embedded within the sporocarp walls in interwoven hyphae that is regularly septate. This hyphae forms an outer and inner zone of pseudoparenchyma between which the spores are enclosed. In TEM the individual septa of these hyphae are typical of ascomycete taxa, with a single central pore plugged with electron opaque material and one or more associated Woronin body (fig. 2). Also, in TEM the spores are shown to have two homogeneous walls, a thin outer and a thick inner, sepatated by a tripartite transition zone. The outer wall appears entirely or partially fused to the surrounding hyphae.

The evidence from light microscopy also indicates that Glaziella is an ascomycete. Within the interwoven hyphae of the middle layer isolated pockets of densely-staining hyphae develop, possibly as a result of hyphal conjugation. Locules form in the centers of these pockets and the young asci develop within the locules. The young asci are clavate with thick, hyaline walls and dense cytoplasm. As they mature a single ascospore develops within. Eventually, the ascal walls disintegrate leaving the individual spores embedded within the sporocarp wall.

Thaxter (1922), regarding Glaziella, stated that "a final opinion with regard to its true relationships can hardly be formed until the true nature of the primary vegetative and sporogenous hyphae is known." We feel that the evidence presented here indicates that the "vegetative" hyphae of the sporocarps is typically ascomycetous and that the "sporogeneous" hyphae actually form young asci, not chlamydospores as was originally presumed. Therefore, we feel that this fungus should be transferred to the Ascomycetes and, since no existing family or order can comfortably accomodate it, in a separate publication we are describing the Glaziellaceae and Glaziellales.

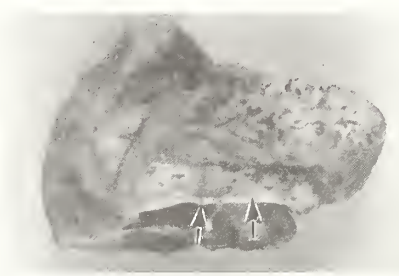

Figure 1. Glaziella aurantiaca. Typical sporocarp broken open exposing spores (arrows) embedded in the thin sporocarp wall. x2.5.

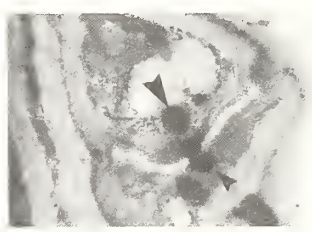

Figure 2. Glaziella aurantiaca. Ascomycetous septum typical of the sporocarpic hyphae. Note plug (small arrow) and Woronin body (large arrow). (bar= 0.5 µm).

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IMMUNOLOGICAL TECHNIQUES IN THE IDENTIFICATION OF ENDOGONACEAE

By

F.E.B. Aldwell, I.R. Hall and J.M.B. Smith

Keywords--Immunological techniques, fluorescent-antibody technique, ELISA

Introduction

Immunological techniques were investigated to determine whether they could be used to measure the rate of spread of elite strains of endomycorrhizal fungi introduced into field soils. However, it was anticipated that if these techniques proved successful they could also be of value as an aid to the taxonomy of the Endogonaceae.

Materials and Methods

External endomycorrhizal hyphae were harvested from infected plants grown hydroponically, in nutrient film culture, or in pot cultures and lyophilized. Details of the preparation of antisera are given in Aldwell *et al.* (1983) and the methods used to apply the fluorescent-antibody technique and the enzyme linked immunosorbent assay (ELISA) are given in Wilson *et al.* (1983) and Aldwell *et al.* (1983) respectively.

Results and Discussion

The immunofluorescence reactions of *Glomus mosseae*, *Gigaspora margarita* and *Acaulospora laevis* antisera with a range of endogonaceous and non-endogonaceous fungi are presented in Table 1. A major difficulty in assessing immunofluorescence was the variability in staining reactions (Table 1). It was concluded that the fluorescent-antibody technique in its present stage of development is suitable only for distinguishing endomycorrhizal fungi from non-endogonaceous species and for distinguishing between some endogonaceous genera.

The performance of one of six endogonaceous antisera with 14 fungal antigens in ELISAs is presented in Figure 1. The height of each bar represents the degree of similarity between antiserum and antigen, i.e., the higher the bar the closer the assumed relationship of each antigen to the homologous species. The data show that with few exceptions the 14 antigens fall into groups which correspond well with the current Gerdemann and Trappe classification with: *A. laevis* and *Gigaspora* spp. being closer than the two are to the *Glomus* spp. and *S. dussii*, and the *Glomus* spp. serologically close to *S. dussii*. The exception to this pattern was *Glomus tenue* which reacted poorly with *G. mosseae*, *S. dussii* and both *Gigaspora* antisera, and only moderately with *G. clarum* and *A. laevis* antisera. This reflects the unique morphology of *G. tenue*. *Pezizella ericae*, *Rhizopus oryzae* and *Mucor heimalis* showed low cross-reactivity with all six antisera though *Mortierella wolfii* was moderately cross-reactive with all six antisera. However, the data are probably not strong enough to say whether this reflects a closer relationship of the Endogonaceae to the Mortierellaceae than to the Mucoraceae.

Table 1. Immunofluorescence reactions assessed on a scale of 0-5 of three endogonaceous antisera with homologous and heterologous fungal hyphae.

	Antiserum			
Antigen	*G.mosseae*	*G.margarita*	*A.laevis*	Type of fluorescence
G. mosseae	4	2	2	Uniform, broken tip
G. tenue	2	1	2	Uniform
G. fasciculatum	3	2	4	Irregular
G. clarum	2	1	2	Uniform
G. macrocarpum	4	1	1	Irregular
Gi. calospora	1	4	2	Uniform
Gi. margarita germ tube hyphae	3	4	3	Uniform, broken tip
Gi. margarita	1	4	3	Uniform, broken tip
A. laevis	3	2	4	Irregular broken tip
S. dussii	3	2	2	Uniform
Pezizella ericae	2	1	1	Uniform
Mortierella wolfii	1	0	0	Uniform
Mucor heimalis	1	0	0	Uniform
Rhizopus oryzae	1	0	0	Uniform

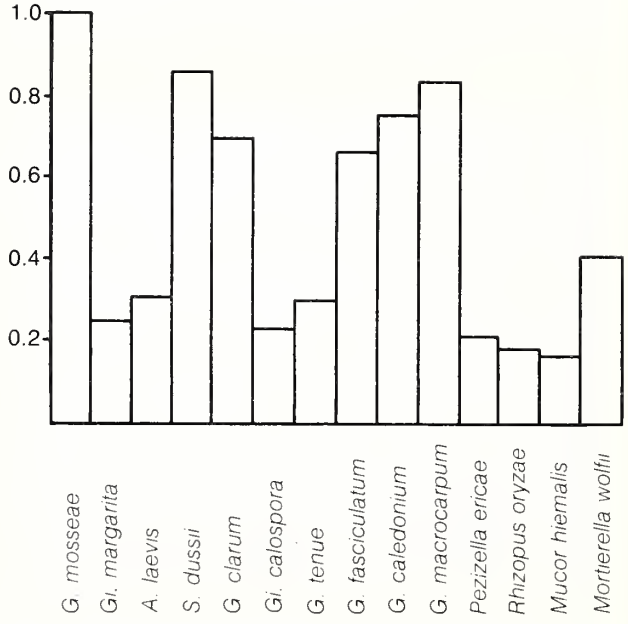

Figure 1. Performance of *G. mosseae* against fourteen fungal antigens using indirect ELISA. LSD (0.1%) = 0.05.

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GERMINATION SHIELDS IN THE GENUS *GIGASPORA*

By

Christopher Walker and Francis E. Sanders

Keywords -- *Gigaspora*, germination, germination shields, compartmentalization

Introduction

There have been two modes of germination reported from spores of the genus *Gigaspora* Gerd. & Trappe. In one, represented by *G. gigantea* (Nicol. & Gerd.) Gerd. & Trappe, the germ tube emerges directly through the spore wall, whereas in the other, represented by *G. calospora* (Nicol. & Gerd.) Gerd. & Trappe, there is formation of a specialised structure from which the germ tubes emerge before passing through the outer spore wall. The former was described in detail by Sward (1981). The latter has not been described in detail, but has been commented upon a number of times (Hall 1977, Sward et al. 1978, Walker & Rhodes 1981). The germination method found in the *G. calospora* type has been described as *chamber formation* or *compartmentalization*, but Koske, Miller & Walker (1982) preferred to call the structure a *germination shield*. We shall refer to the form found in *G. gigantea* as *direct germination*, and we will retain the phrase *germination shield formation* to refer to the *G. calospora* type of germination.

In addition to possessing distinctive modes of germination, the two groups within *Gigaspora* have other differences. The wall types (as described by Walker (1983)) are different. Spores in the *gigantea* grouping do not have membranous walls. Instead, they appear to have only a single wall group consisting of unit- and laminated- walls. The *calospora* group have, without exception, at least one distinct membranous inner wall. Besides this, the former group always have spiny or papillate auxiliary cells (Trappe & Schenck 1982), whereas those structures on the latter grouping are invariably smooth in youth, becoming knobby when mature.

It seems, from initial study of several different species, that the morphology of the germination shields will have taxonomic value, though this will be limited because they develop late in the life of the spore, and therefore cannot be found on immature specimens.

Germination shield formation seems to begin by production of a hole through a membranous wall. The pore contents, presumably surrounded by plasmallema, then balloon through this hole, but are constrained by the outer wall layers. Wall material is then laid down, continuous with the original membranous wall, around the bulging membrane, and invaginations of the wall develop to form a chambered structure. In the simplest form, this happens only once, and there are two germ tube initials formed. This simple form is found in *G. heterogama* (Nicol. & Gerd.) Gerd. & Trappe. In some species, there are apparently several consecutive phases of development in which growth of the germ shield, and thickening of its walls, is followed by a resting stage which, in turn, is followed by another burst of growth. This can be repeated several times, resulting in a very complex germination shield, with up to ten pairs of germ tube initials. This is well illustrated in *Gigaspora nigra* Redhead in Nicol. & Schenck. The germ tube initials seem always to appear in pairs, and although only a few observations have been made on specimens from which germ tubes have actually emerged, it seems that only one of each pair germinates.

We believe that these differences between the two groups of spores are fundamental, and we consider that the genus *Gigaspora* should be separated into two genera, though we do not propose formally to do so here. The one containing *G. gigantea* that lacks germination shields would remain as *Gigaspora*. The other group would be placed in the new genus (which we propose later to call *Scutellospora*). The two putative genera are readily separable. The germination shield can usually be found on at least some specimens in any reasonably large collection, although some species form them more readily than others. The auxiliary cells are distinctly different, and, although these may not be found in field-collected material, they can readily be retrieved from pot cultures. Even if neither of these structures can be found, their existence can be inferred from the wall structure of the spores. Where membranous walls are present, both germination shields and knobby auxiliary cells will occur. Where spores have walls in a single group, lacking membranous walls, then germination will be by direct regrowth, and the auxiliary cells will be spiny or papillate.

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AN UNDESCRIBED ACAULOSPORA FROM ARIZONA

By

C.M. Pfeiffer, Christopher Walker, & H.E. Bloss

Keywords--Acaulospora, Sudan grass

Introduction

This species was originally isolated from a bed of sand, in the glasshouse at Tucson, AZ, used for rooting stem cuttings of various ornamental plants.

Methods and Materials

Pure cultures were obtained from mixed stock culture by selecting 5-10 azygospores and placing them in sterilized sand planted with Sorghum sudanense (Pip.) Stapf, Sudan grass, maintained in a growth chamber at a diurnal temperature range of 15-27°C. Light was provided by incandescent and fluorescent lamps for 16 hr daily.

A core sample of sand was removed after 90 days and observed microscopically for infection of roots and production of new azygospores. All stages of fungal development were observed using standard procedures for scanning electron and light microscopy.

Results and Discussion

Sporocarps unknown. Azygospores formed singly in the soil or occasionally within roots, borne laterally on a smooth unbranched hyphal cell which terminates in a globose to subglobose saccule (vesicle). Spores globose to obovoid (80.0-) 87.5-102.5 (-105.0) X (80.0-) 92.5-105.0 (-107.5)µm with a smooth laminated outer wall 2.5-3.5 µm thick and an ornamented membranous inner wall 0.75-1.25 µm thick which readily separates upon crushing the spore. The species is routinely maintained in pure culture on roots of various host plants.

Figure 1. Azygospore with outer laminated wall and inner membranous wall with ornamentation. (light micrograph)

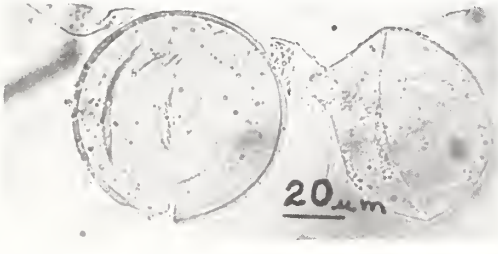

Figure 2. A developing azygospore with attached saccule. (light micrograph)

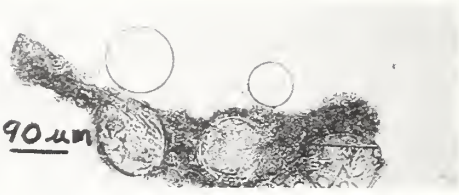

Figure 3. Three azygospores which developed within a senescent rootlet of Sudan grass similar to the mode of azygospore production by Acaulospora trappei Ames & Linderman. (light micrograph)

Figure 4. Vesicles and hyphae formed in a rootlet of Sugan grass. (light micrograph)

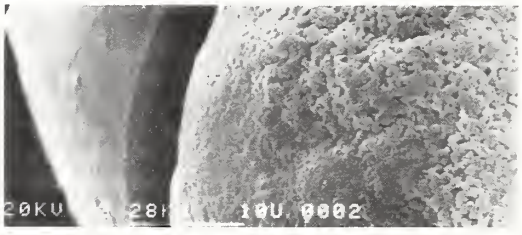

Figure 5. Electron micrograph illustrating the smooth, laminated outer wall and granular ornamention on the surface of the inner wall.

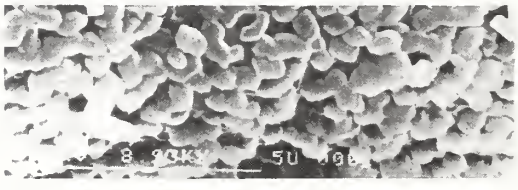

Figure 6. Electron micrograph showing detail of the granular ornamentation of the inner wall.

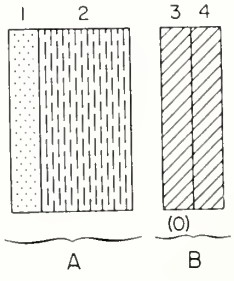

Figure 7. MUROGRAPH.

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IDENTIFYING ISOLATES OF *LACCARIA* USING CULTURAL AND MATING STUDIES

By

Gregory M. Mueller and Nils Fries

Keywords--Tissue culture, basidiospore germination, dedikaryotization, somatic culture mat analysis

Introduction

A detailed examination of somatic culture mats has been undertaken during an ongoing taxonomic and biological study on the genus *Laccaria* Berk. & Br. (Mueller, 1984; Fries and Mueller, 1984). Fries and Mueller (1984) also examined the utility of employing mating studies to determine the degree of intra- and interspecific genetic isolation in *Laccaria*. These studies yielded information which facilitated species identification as well as data which further characterized and delimited taxa of *Laccaria*.

Methods and Materials

Somatic culture mat analyses were performed on 74 isolates representing 10 *Laccaria* species from Canada, Sweden, and the U.S.A by incubating them for 6 wks on each of three to four media using the methods outlined in Mueller (1984). The media employed were: Difco potato dextrose agar (PDA), modified Melin-Norkrans medium (MNM), 2 % malt extract agar (MA), and recently, the synthetic medium of Fries (N6:5) (Fries, 1978). The mating studies followed the techniques outlined by Fries (1983) and Fries and Mueller (1984). Dedikaryotization of dikaryotic mycelium was attempted following the procedures of Kemp (1974) and Leal-Lara and Eger-Hummel (1982).

Results and Discussion

By using somatic culture mat analysis it was possible to identify to species or species group all of the examined isolates. Isolates of *L. amethystea-occidentalis* Mueller, *L. nobilis* Smith apud Mueller, *L. oblongospora* Mueller, *L. ochropurpurea* (Berk.) Pk., and *L. trichodermophora* Mueller displayed unique culture mat morphologies while isolates of the other species could only be identified with certainty to one of two species groups (1. *L. amethystea* (Bull.) Murrill and *L. bicolor* (Maire) Orton and 2. *L. altaica* Singer, *L. laccata* (Scop. : Fr.) Berk. & Br., and *L. proxima* (Boud.) Pat.). This discrimination of species was only possible when the isolates were grown on several media. Isolates grown on PDA usually showed the greatest interspecific differences but in some cases the distinguishing characteristics were observed on MNM or N6:5. Isolates grown on MA showed little species specific variation.

Data from preliminary mating studies using North American material have supported Fries and Mueller's (1984) report of a high correlation between mating groups and species based on basidiocarp morphology. To date only isolates of *L. laccata* have been found to form more that one mating group and the other species are genetically isolated from one another.

Since the monokaryotic isolates required for mating studies can be formed by dedikaryotizing dikaryotic isolates obtained from tissue culture or isolated from mycorrhizae, mating studies will be useful for identifying cultures which lack voucher specimens or viable basidiospores once more efficient dedikaryotization techniques are developed. Neither of the methods employed routinely dedikaryotized *Laccaria* isolates.

As mentioned by Miller *et al.* (1983) and Pantidou *et al.* (1983), our knowledge of cultural characters in the Agaricales is very limited. These extensive studies on *Laccaria* have demonstrated the usefulness of such cultural and mating studies for taxonomic and biological investigations. A great need exists, however, to develop standardized techniques for culture mat studies. Until workers in various laboratories agree upon the media and incubation regime employed, results of such studies will not be comparable.

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ULTRASTRUCTURE OF ENGLISH OAK MYCORRHIZAL CYSTIDIA

H. H. Edwards and R. V. Gessner

Introduction

In a previous study (1) one of the most unusual structures to be described was a cell type that projects from the fungus mantle. This structure is called a cystidium. The ultrastructure of cystidia from ectomycorrhizae had not previously been described. However, there have been a couple of scanning electron microscope reports of ectomycorrhizal cystidia published (2,4). This report will describe the TEM of oak ectomycorrhizal cystidia.

Materials and Methods

Mycorrhizal short root tips of English oak were processed for TEM using the procedure of Mueller & Greenwood (3).

Results and Discussion

The cystidia occur as the outermost layer of the mantle and they are the portion of the ectomycorrhiza that directly interfaces with the rhizosphere. The cystidia have a morphology very much different from the other mantle cells. Mantle cells are typically cells interwoven to form a loose or dense mesh; cystidia are terminate cells having a flask-shape and terminated by a knob. Large numbers occur on the surface; often times being stacked one next to the other. Mantle cells are embedded in slime material. However, the cystidia project out through the slime into the rhizosphere.

There are two distinct layers to the cystidial wall. The outer layer is thin and in the TEM is quite electron dense but grainy. The inner wall layer is more homogeneous in electron density and much thicker. However, the thickness is often uneven and the inner surface frequently has a wavey surface. The two layers of the wall are sometimes separated. Cystidia are attached to mantle cells and are connected by a dolipore septum. More than one cystidium may be attached to the same mantle cell. In the soil cystidia penetrate into the soil debris and thus interact directly with the rhizosphere.

Cystidia are binucleate with the nuclei situated at base of the cell. Mitochondria are present and are displayed parietally with the majority in the lower half of the cell. The most conspicuous structure internally are the electron dense blobs that occur mainly in the upper portion of the cell. The blobs have a granular texture. Usually just below the blobs are areas where diffuse granular material may be coalescing to form the blobs. Associated with these areas and with blobs are membraneous material. These bits of membraneous material may also be found in Hartig net cells.

Another substance found in the cystidia has a more amorphous texture than the blobs. This amorphous material occurs throughout the cystidium. The material may occur as spheres or stretch-out with no regular shape.

The tip of the cystidium is enlarged into a knob. The knobs have considerable variation in their internal structure, however, all have the outer wall layer. Internal contents of the knob appear to be pushed up from the lower portion of the cell. Granular blobs are found in some of the knobs, in others amorphous material occurs, and in others the inner wall layer that occurs in the lower portion of the cell. This gives the impression that the knobs are constantly changing their composition. Such a situation would occur if the knobs were involved in secretion of materials through the outer wall layer. In support of this idea, amorphous-like material can be seen in amongst the rhizosphere debris in those portions of the ectomycorrhiza in which the soil debris was not removed during tissue preparation. It is postulated that the secretory products play a role in prohibiting insect grazing.

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ECTO-, ECTENDO-, AND PSEUDOMYCORRHIZAE OF DEMATIACEOUS FUNGI WITH PINUS RESINOSA

By

H. E. Wilcox and C. J. K. Wang

Keywords--Pinus resinosa, Dematiaceous fungi, mycorrhizae, pseudomycorrhizae

Introduction

The name Mycelium radicis atrovirens Melin was proposed by Melin (1923) for a coarse, brown non-mycorrhizal fungus isolated from the roots of pine and spruce trees growing on drained peat bogs. The infections from this fungus were characterized by persistent intracellular hyphae, but formed no Hartig net or mantle. Melin coined the further term "pseudomycorrhizal" for such infections.

The concepts of Mycelium radicis atrovirens and pseudomycorrhiza have been interpreted in various ways by subsequent investigators. Much confusion has arisen because many of these interpretations have differed markedly from Melin's original formulations.

Recent investigations of M. r. atrovirens have served to dispel some of this confusion. Richard and Fortin (1973) studied 41 strains of the fungus and found that 15 of them possessed conidiophores, phialides and conidia of Phialocephala dimorphospora. They attributed the pseudomycorrhizae of Picea mariana to this species. Kowalski (1973) investigated strains of M. r. atrovirens from mycorrhizal roots of Scotch pine and found that they could be divided into two groups, one group forming ecto-mycorrhizae.

Results and Discussion

In our mycorrhizal research, a number of dark sterile fungi resembling M. r. atrovirens were isolated from roots of pine. During subsequent cold treatment of these sterile cultures for six months to a year, three were found to produce conidia which enabled us to elucidate the conidiogenesis of these fungi and to propose the following new species: Phialophora finlandia, Phialocephala fortinii, and Chloridium paucisporum. Thus three cultures among the many isolates of M. r. atrovirens represent members of three different genera of Hyphomycetes.

Our investigations also confirm those of Kowalski (1973) that not all isolates of M. r. atrovirens are pseudomycorrhizal. Two of our three fungi formed ecto- and ectendomycorrhizae in Pinus resinosa and the third was pseudo-mycorrhizal. Descriptions of these are presented in our poster, and comparisons are made with root associations formed by the ubiquitous black fungus, Cenococcum geophilum, to which they have resemblances. Effects of these dematiaceous fungi on seedlings grown at pH3 and pH5 in mycorrhizal synthesis experiments are also shown on the poster.

Methods and materials of these experiments are being presented in Mycologia in a paper on these three new fungi and are not presented on the poster. The poster summarizes the sporulating characteristics of each fungus and shows details of its association with the roots of Pinus resinosa.

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SCLEROTIUM DEVELOPMENT IN TWO ECTOMYCORRHIZAL FUNGI

By

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Keywords--Pisolithus tinctorius, Paxillus involutus, histochemistry.

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Introduction

Sclerotia of pathogenic fungi have been studied extensively (Willetts, 1972) so that considerable information exists concerning their ontogeny and structure. In contrast, although sclerotia of ectomycorrhizal fungi have been isolated from soil and some preliminary observations on the ontogeny and structure of Pisolithus tinctorius sclerotia have been published (Piché and Fortin, 1982), there is little detailed information available on sclerotia of any ectomycorrhizal fungi. The objectives of the present study are, therefore, to study the ontogeny and structure of sclerotia of two ectomycorrhizal fungi, Pisolithus tinctorius and Paxillus involutus produced in association with pine seedlings grown in plastic growth pouches.

Materials and Methods

Cultures of Pisolithus tinctorius Coker and Couch and Paxillus involutus (Batsch) Fr. were maintained on modified Melin-Norkrans (MNM) agar medium (Fortin et al., 1980). Plugs of mycelium (10 mm in diameter) were taken from actively growing cultures and transferred to fresh medium for 3 days to permit regeneration of hyphae on margins of the plug. Plugs were then transferred to plastic growth pouches containing seedlings of Pinus strobus L. and Pinus resinosa Ait. (Fortin et al., 1980). Four or five plugs were placed in each pouch, about 5 mm. from actively growing lateral roots. 10 ml of MNM nutrient solution was added to the pouch at this time. After mantle formation a further 10 ml of nutrient solution was added. Sclerotia of both species of fungi formed within the pouch adjacent to lateral roots. Sclerotia of P. involutus also formed in pure culture.

Sclerotia prepared for light and transmission electron microscopy, were fixed in 2% glutaraldehyde in Sorensen's buffer, postfixed in osmium tetroxide, dehydrated in acetone and gradually infiltrated with Spurr's resin. Whole sclerotia prepared for scanning electron microscopy were fixed in 4% glutaraldehyde in Sorensen's buffer, washed and treated with 1% thiocarbohydrazide, washed and refixed in 1% osmium tetroxide. Sclerotia were then dehydrated in a graded acetone series followed by critical point drying. Sclerotia prepared for histochemistry were fixed in 4% glutaraldehyde followed by dehydration in the following sequence: 2 - 12 hour changes in each of methyl cellosolve, n-propanol, n-butanol and ethanol. Tissues were infiltrated over a period of two weeks at 4°C with increasing concentrations of glycol methacrylate.

Results and Discussion

Fungal strands of P. tinctorius turned a bright yellow prior to initiation of sclerotia as described by Piché and Fortin (1982). Small hyphal branches grow at right angles to the strand, branching frequently and forming a lenticular swelling on the strand. As sclerotia approached maturity, hyphal tips became swollen, ceased to grow and formed a darkly pigmented rind. Mature sclerotia consisted of a thick-walled outer rind, an outer cortex of thick-walled cells, an inner cortex containing numerous metachromatic granules and a filamentous medulla within a matrix. Histochemical studies showed that walls, matrix and much of the cell contents consisted of polysaccharides, especially glycogen. Protein and lipid bodies were also present in cortical and medullary cells.

Initiation of P. involutus sclerotia occurred at hyphal tips which branched and proliferated, forming small tangles of hyphae. An immature sclerotium appears as a white cluster of hyphae, usually forming adjacent to roots. Hyphae continue to grow around the developing sclerotium until it matures. Exudates are common on the surface of the sclerotium. At maturity, a black rind forms beneath an outer hyphal weft. Mature sclerotia consist of a one to two celled rind of thick-walled cells, a narrow cortex and a large central medulla of thin-walled, pseudoparenchymatous tissue with little intercellular matrix.

Histochemical tests indicate cell walls consist partially of polysaccharides. Cell contents are mainly glycogen with large protein bodies and smaller lipid deposits also present.

The structure and cytochemistry of these sclerotia indicate that they play a persistence role. Further field studies may indicate their role in providing inocula in natural and man-made forests. Sclerotia also offer a potential source of inoculum for establishment of ectomycorrhizal nursery seedlings.

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SUSPECTED ECTOMYCORRHIZAL FUNGI COMMONLY ASSOCIATED WITH DIPTEROCARP SPECIES

By

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Keywords — Dipterocarp, Ectomycorrhiza, Agaricus, Amanita, Boletus, Cantharellus, Geastrum, Lactarius, Russula, Scleroderma

Introduction

The significance of mycorrhiza in the growth of trees in tropical forest have long been recognized (Bowen, 1980). It has been observed that some mycorrhiza exists on some of Dipterocarp species (Setiabudi, 1980; Nuhamara, 1984). Additional observations on the presence of mycorrhizae were carried out mainly on the identity of the fungal symbionts associated with different Dipterocarp species. The informations gathered would be useful for studies on the regeneration of the Dipterocarp forest in Indonesia.

Methods and Materials

Periodical observations on mycorrhiza were mainly made on a 40 year old plantation of Dipterocarp species in West Java, and on Dipterocarp natural forest in East Kalimantan. During the observations fruiting bodies of the suspected fungal symbionts of Dipterocarp species encountered on the forest floor were collected for identification. Cross section of mycorrhizal roots of Dipterocarp species were made. The morphological characteristics and the microscopic features of each mycorrhizal association were recorded. Identification of the fungal symbiont was carried out for each mycorrhizal association.

Results and Discussion

Twenty two Dipterocarp tree species growing in plantation as well as in natural forest were observed to form mycorrhizae. Different species of Agaricus, Boletus and Gastromycetes are associated with these different Dipterocarp species. A tree species may be able to form mycorrhiza with different fungal species. This can be seen among others on Shorea stenoptera, S. leprosula, S. pinanga, S. selanica, and Hopea bancana.

Various combinations between different Dipterocarp and fungal species were found in different locations. The mycorrhizal branching on different tree species may be simple to somewhat coralloid or pyramidal and white, yellow or brown in color. The surface may be smooth with or without loose weft of hyphae. The Hartig net developed well on all Dipterocarps observed. A typical repeated or double mantle formation was observed on S. stenoptera associated with Russula delica or with another unidentified fungal species.

Dipterocarpus carnatus, Dryobalanops oocarpa, Hopea rudiformis and Shorea ovalis were found to be associated with ectomycorrhizal fungi. However, the fungal symbionts were not identified yet.

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ECTOMYCORRHIZAL SPECIES OF AGARICS FROM SOUTH INDIA.

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KEYWORDS - Pinus patula, agaric species

Introduction

Systematic study of the members of Agaricales is neglected in India when compared to other groups of fungi. The number of Agarics reported from South India comprising the four states of Tamil Nadu, Kerala, Karnataka and Andhra Pradesh is still less. Even though the first report of an Agaric in India was from South India (Montagne, 1842), only 10 species belonging to 8 genera were added to the South Indian list in a period of 132 years up to 1974. Out of these, none of them are mycorrhizal species. To fill this gap serious taxonomic work on the members of this group occurring in South India was taken up (Natarajan and Raman, 1983). Bakshi (1966, 1974) has studied the Mycorrhizae of Eucalyptus and some gymnosperms in North India. The introduction of Pinus and Eucalyptus in South India for various purposes increases the need for studying the ectomycorrhizal species of Agarics associated with them. So special attention is given for the enumeration of mycorrhizal species associated with Pinus particularly Pinus patula.

Methods and Materials

Pinus patula plantations present in Kodaikanal and Nilgiris of Tamil Nadu planted by Tamil Nadu Forest Department were selected for regular observation for the agarics. Agarics present in these plantations were collected. Field characters such as the colour and size of the pileus, stipe and lamellae, presence or absence of annulus, etc., were noted from the fresh specimens. The colour terminology used is that of Kornerup and Wanscher (1967). The specimens were dried for further microscopic studies. The dried specimens were revived with 10% KOH solution before freehand sections were made. Stains such as 1% aqueous Phloxine, Congo red solution or cotton blue were used. Melzer's reagent was used to study the amyloidity of spores and other tissues. Cresyslblue solution was used to study the metachromatic reaction of spores.

Fruitbodies growing in close association with the roots were collected and their mycelial connections were traced with the help of a knife. Mycorrhizal roots were removed by washing in running water. Using Zak's (1973) key for identification, the morphological types of mycorrhizae were differentiated.

Results and Discussion

Out of the many specis identified, the following species were found to be mycorrhizal: Amanita muscaria, Laccaria laccata, Suillus brevipes, S. pallidiceps, S. punctatipes, S. subluteus, Russula parazurea, Thelephora terrestris, Scleroderma citrinam and Lycoperdon perlatum. In Russula parazurea and Lycoperdon perlatum corralloid type of mycorrhizae were found whereas in all other cases bifurcate type wer found.

It is observed that in newly introduced plantations Scleroderma citrinam, Suillus spp. were dominant, in middle aged plantations Laccaria laccata, Thelephora terrestris and Russula parazurea were dominant whereas in older plantations Amanita muscaria and Lycoperdon perlatum were dominant.

Laccaria ohiensis is found to be present only in Eucalyptus plantations and absent in Pinus plantations.

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CHARACTERIZATION OF VESICULAR-ARBUSCULAR ENDOMYCORRHIZAL FUNGI FROM PEANUTS IN THE PHILIPPINES

By

Lina L. Ilag and Ruth A. Taber

Keywords--Arachis hypogaea, mycorrhizae, groundnuts, Glomus, Sclerocystis

Introduction

Peanut (Arachis hypogaea L.) is locally known in the Philippines as "mani". Local production is insufficient to meet demand in the country. In 1980 ca 50,000 ha were planted to peanuts and production totalled ca 50,000 MT. To supplement local production, 10,730 MT of peanuts were imported. Cooperative research programs are underway to study the value of indigenous VAMF for the growth of peanuts both in current production areas and in other areas that may in the future be planted to peanuts. The influence of VAMF on other crops, both in rotated and intercropped systems, will be important factors to be considered. Such crops include sugarcane, mungbean, soybean, cassava, coconut, papaya, citrus, and rice.

This study focused on characterization of spores associated with numerous crops, but especially as related to the peanut.

Materials and Methods

Peanut soil and root samples were collected from Cotabato, Albay, Los Banos, Isabela, Cagayan, and Zambales during 1983. Soils planted to other crops were also sampled at Cebu, Davao, Ilocos Norte, La Union, Binangonan and Luisiana. Soils were collected adjacent to roots and 100 g of a composite sample were wet sieved (Gerdemann and Nicolson, 1963). Spores were either backwashed into separatory funnels and collected on filter papers or centrifuged in sucrose solutions. Collected spores were observed in lactophenol and/or water.

Results and Discussion

Spore counts conducted on 16 soils planted to peanuts, rotated to peanuts or considered for peanut production, revealed that the peanut soil from San Marcelino, Zambales contained the highest numbers of spores (Table 1). Spores had been collected 38 days after sowing Pn-2 peanuts at this site. Cropping pattern consisted of peanut, sweet potato, tomato, peanut. The soil was a sandy loam with a pH of 6.0. Glomus multicaule, G. monosporum, G. mosseae, G. microcarpum, Sclerocystis rubiformis, S. sinuosa and Gigaspora spp. were found at this site. Although soils at Davao (Banana) also contained many spores, there was less diversification of species. Glomus species predominated in these soils. Glomus multicaule was present, as well as G. mosseae. Evidence for the presence of G. intraradices was observed in cleared roots, but no extramatrical spores were found. Spores typical of G. caledonium and G. convolutum were observed at Cagayan in soils previously planted to peanuts. Many deteriorated and/or dead spores were also found. Such spores were unidentifiable. Gigaspora margarita is documented from the Laguna area where dead spores were found after rice. Sclerocystis coremioides, S. sinuosa and S. rubiformis were found on the island of Cebu in soils associated with corn. Lowest spore counts were obtained in the rice, corn, and peanut fields under higher water regimes. Spores sieved from such fields, particularly with and after rice, were deteriorated and in many cases, unidentifiable. Low populations were found in Laguna under coconut, in Cagayan and Albay in peanuts, in Cebu planted to corn, Putho in weeds, and Catabato in cowpea. An unexpected variety of species has been observed associated with peanuts (Stichler, Pettit, and Taber 1972, Nicolson and Schenck 1979, Taber 1984) and other crops in the Philippine Islands and these species are now being obtained in pot culture.

Table 1. Soil Evaluation for Presence of VAMF Spores

Location	Crop	VAMF Spores
Cebu	corn	low
Putho	portulaca	low
Cotabato	peanut	moderate
Davao (SABA)	banana	high
Davao (Latundan)	banana	moderate
Cotabato (MSU)	cowpea	low
Ilocos Norte	cowpea	moderate
Albay	peanut	low
Putho, Los Banos	peanut	moderate
Isabela	peanut	moderate
Cagayan	peanut	low
Binangonan	weeds	moderate
Luisiana, Lag.	coconut	low
San Marcelino	peanut	high
IRRI, Los Banos	rice, corn, peanut	none-low
La Union	mungbean	moderate

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CHARACTERIZATION OF VESICULAR-ARBUSCULAR ENDOMYCORRHIZAL FUNGI FROM PEANUTS IN THAILAND

By

O. Nopamornbodi, Y. Vasuvat, and Ruth A. Taber

Keywords--Arachis hypogaea, mycorrhizae, Glomus Sclerocystis, Acaulospora, Gigaspora

Introduction

Peanut production is being promoted in Thailand, especially in the Northeastern part of the country. The soil in NE Thailand is low in phosphorus and the climate is very dry. For these reasons, the NE provinces should be more suitable for peanut cultivation than many other crops.

This study was undertaken to collect spores of VAM fungi for identification of indigenous species associated with peanuts and to establish pot cultures of prominent species. The goal of this work is to eventually inoculate peanuts in the field in Thailand with superior strains and to introduce other known species from other parts of the world that might also be effective. It is expected by this means that Thai farmers will realize increased peanut yields, better quality peanuts will be available to the populace, lower grade and cheaper phosphorus sources may be utilized effectively, and this information may be put to use in other peanut growing areas of the world.

Materials and Methods

Soil and root samples were collected from several areas in peanut fields from May 1983 to April 1984. In each peanut field, five soil samples were randomly collected at 0-15 cm depth and composited. One hundred grams of soil from each composite sample were taken from wet sievings as described by Gerdemann and Nicolson. After sieving, fractions retained on 450, 250, 90, and 63 micron sieves were observed for VAM spores in 9 mm petri plates under a dissecting microscope (X 20-70). Spores of mycorrhizal fungi were removed from the detritus with pipettes and transferred to Ringer's solution. Spores and sporocarps were mounted in lactophenol on glass slides for identification. Taxonomic keys used for species identification included Trappe's synoptic key and various original papers in the literature.

Results and Discussion

This report constitutes a preliminary survey on VAMF associated with peanuts in Thailand. Characterization in some cases was based on observations of only 1 or 2 spores sieved from the soil. Numbers of VAMF spores in soil at various locations varied from 0 at Pisanulake (Alfisols, pH 4.5 - 5.7) and Makornsajsima (Inceptisols, pH 4.0 - 4.5) to over 300 spores/g at Lampang (Alfisols, pH 6.6 - 7.7). None of the soils contained more than 43 ppm available P and most of the soils were low in P. See Table 1. VAMF fungi representing four genera were observed in soils. Glomus species were the most frequent species observed. Identifiable species included Glomus mosseae (Nicol. & Gerd.) Gerd. & Trappe, from Saraburi province, G. multicaule Gerd. & Bakski, G. melanosporum Gerd. & Trappe, G. microcarpum Tul. & Tul. from Lampang, and one other Glomus spp. from Tak. Acaulospora species included Acaulospora scrobiculata Trappe, and one other Acaulospora sp. with prominent ornamentations connected by delicate membranous material. Other spores resembling Acaulospora spores were occasionally observed; however limited material prevented accurate documentation. Representatives of the genus Sclerocystis included Sclerocystis coremioides Berk. & Broome, S. sinuosa Gerd. & Bakshi, and S. clavispora Trappe. Gigaspora margarita Becker & Hall and G. gregaria were found at Lampang: an unidentified Gigaspora with a warty dark wall enclosed by hyaline sloughing material was also observed. Currently attempts are being made to obtain all species in pot culture.

Table 1. Soil Characteristics and Spores Retrieved from Different Locations

Location[1]	Soil pH	P ppm	Spores/ 100 g
Lampang (A)	6.6-7.7	17-26	240-375
Chiengmai (A)	6.4-6.5	37-39	245-270
Kalasin (U-A)	4.6-5.0	7-14	54-340
Nakornsajsima (I)	4.0-4.5	11-13	0
KhonKaen (UT)	6.3-6.6	9-10	27-63
Mahasarakarm (UT)	4.5-5.4	5-7	120-173
Roi-et (UT)	4.7-5.4	11-22	46-74
Chumporn (UD)	6.1-6.2	37-43	127-134
Pisanulake (A)	4.5-5.7	33-39	0
Kabinburi (US)	6.9-7.7	16-22	20-168

[1] Letters refer to soil orders: A=Alfisols, U-A=Ustults/Aquults, I=Inceptisols, UT=Utisols, UD=Udults, US=Ustults

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ULTRASTRUCTURE OF PARASITISM OF VESICULAR -ARBUSCULAR MYCORRHIZAL FUNGI BY PHLYCTOCHYTRIUM PLURIGIBBOSUM

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Keywords--Mycoparasitism, Glomus clarum, host-parasite interactions.

Introduction

Parasitism of chlamydospores or azygospores of vesicular-arbuscular mycorrhizal fungi (VAMF) by Palyctochytrium occurred in the rhizosphere of soybean and peanut (Ross and Ruttencutter, 1977). This case of parasitism was suggested to be realted to decline of population of VAMF in nature. Besides, Phlyctochytrium, VAMF were also vulnerable to the attack of Humicola fuscoatra Traaen and Anguillospora pseudolongissima Ranzoni (Daniels and Menge, 1980). In Taiwan, parasitism of VAMF by P. plurigibbosum endemically occurred in rhizosphere of corn. Present communication, by using the P. plurigibbosum / VAMF model, to elucidate the host-parasite interactions at the ultrastructure level.

Materials and Methods

To determine the hyperparasitic activities of the chytrid on VAMF, chlamydospores of Glomus mosseae (Nicol. & Gerd.) Gerdeman and Trappe, G. clarum, and Glomus sp. (#BNa) were surface -sterilized with 2% sodium hypochlorite for 2 min, rinsed with sterile distilled water for three times, and transferred to sterile pond water in a small petri dish. Culture discs of the Palyctochytrium growing in M-3 medium for 1-2 weeks were added to the dishes. Agar discs of M-3 medium added to the dishes containing VAMF spores were used as control. The inoculated dishes were incubated at room temperature. Observation of the hyperparasitism were made at 12, 24, 48, 72h and one week. The heavily infected VAMF spores were prepared for transmission and scanning electron microscopy.

Results and Discussion

Glomus clarum, G. mosseae, and Glomus sp. (#BNa) becamed heavily infected after inoculation with Palyctochytrium plurigibbosum for 24-72h (Fig. 1). P. plurigibbosum was reisolated from the three infected host spores (Fig. 2). In control, no infection occurred during this period. The parasite apparently did not show host specificity at least in the genus level. The mode of infection through artifical inoculation was identical to those occurring in nature, involving the encystment of the zoospores on the host spore surface or its subtending hyphae, formation of appressorium, infection tube, and endobiotic apophysis and rhizoid (Fig. 3). Apophysis and rhizoid were thin-walled, characterized by multivesicular and tubular structures, occasionally surrounded with electron-dense collar, which presumably originated from the host secrection and deposition in response to the infection as a defense mechanism (Fig. 3). The infected host cell wall filled and expanded by the thalli of the parasite and showed partial degradation and disintegration. The parasite caused the death of the host by deprivation of its cytoplasmic contents and disintegration of the cell wall. The nourished epibiotic cysts developed into zoosporangia which discharged zoospores and initiated a new infection cycle. From present investigations, we endorse the suggestion of Ross and Ruttencutter that P. plurigibossum might restrict the population of VAMF in soil, deter the normal function of the endomycorrhizae and downgrade the symbiosis.

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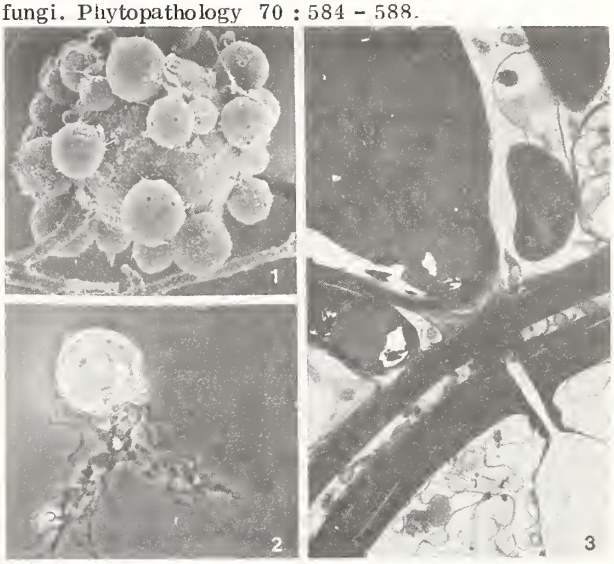

Fig. 1. Clamydospore of Glomus clarum infected and attached with numerous zoosporangia of the hyperparasite, Phlyctochytrium plurigibbosum. X 156.
Fig. 2. Zooaporangium of P. plurigibossum with apophysis and rhizoid viewed under the phase-contrast light microscopy. X 640.
Fig. 3. Infection tube of P. plurigibossum penetrating through the cell wall of G. clarum, and forming apophysis and rhizoid. Penetrated through, electron -dense apposisation zone just locating underneath the plammamembrane hints the defense mechanism in vain. X 3600.

ENDOPHYTES OF EUCALYPTUS

By

M.M. Schoeneberger

Keywords--Eucalyptus regnans, seedling growth

Introduction

It has been demonstrated that mycorrhizae play an integral role in tree nutrition and that Eucalyptus spp. form mycorrhizae. Eucalypts are considered to be predominantly ectomycorrhizal although field investigations and recent experimental evidence (Malajczuk et al.,1981) suggest endomycorrhizae may also be prevalent. As the two mycorrhizal types are quite different structurely, it follows that the roles played by each in host plant nutrition may also be different.

Objectives:
1) to determine the occurrence of endo- and ectomycorrhizae on E. regnans in forest soils,
2) to determine the early growth effects of endo- and ectomycorrhizae on E. regnans.

Objective 1 was pursued via a greenhouse bioassay using soils collected from the Central Plateau Region in New Zealand. The effect of coplants on mycorrhiza formation was also investigated in the bioassay. Objective 2 was resolved using known ecto- and endomycorrhizal symbionts of E. regnans.

Methods and Materials

Study 1--Composite soil samples were collected at 8 sites in Whaka Forest, N.Z. (Table 1). Pots filled with soil from each site were sown with 2 E. regnans seeds each and one of the following coplants: none, clover, rye grass, and radiata pine. Plants were grown for 4½ months. Roots were harvested for mycorrhizal assessment.

Study 2--Pots containing acid-washed sand were sown with E. regnans seed (1/pot) and received one of the following: CONTROL (Filtered washings (FW) from the endo- and ectomycorrhizal inoculum but no inoculum), ENDO (spores of Gigaspora margarita + FW from the ectomycorrhizal inoculum), and ECTO (hyphae of Laccaria laccata + FW from the endo-mycorrhizal inoculum). Seedlings were grown for 4 months at which time they were harvested and measured for biomass.

Results

Study 1--Eucalypt seedlings formed endomycorrhizae in all soils except YD (Table 1). Soils from RB and YP which had experienced the most recent site disturbances produced E. regnans seedlings and coplants with the least endo- and ectomycorrhizae. All coplants formed mycorrhizae to varying degrees No trends were observed regarding coplant influence on endomycorrhiza formation.

Study 2--At 4 months, seedlings possessed ecto- and endomycorrhizae depending on inoculation treatment. No mycorrhizae were observed on control seedlings. Seedlings inoculated with L. laccata were significantly taller and produced greater biomass than control and endomycorrhizal seedlings (Table 3). Seedlings inoculated with G. margarita produced greater heights and weights than control seedlings. These differences were significant at $p < 0.10$ level. The effect of both types of mycorrhiza was to produce an overall larger seedling without changing the proportions of the individual plant components (stem:leaves:roots).

Conclusions

Seedlings of E. regnans were found to readily form both endo- and ectomycorrhizae in a number of N.Z. forest soils. The type and quantity of mycorrhizae formed were deteremined by previous site history and host plants occupying the sites. Although Study 2 only utilized one symbiont species per type of mycorrhiza, the growth differences observed indicate the two types may be affecting host plant nutrition differently as well as other aspects such as disease resistance and drought tolerance.

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TABLE 1. SOIL COLLECTION SITES USED IN GREENHOUSE BIDASSAY.

SITE	TREE SPECIES	YEARS SINCE ESTABLISHMENT
EP---EUCALYPT/PINE	(E. REGNANS & P. RADIATA)	5
UE---UNDER EUCALYPT[1]	(E. REGNANS & P. RADIATA)	5
OL---OLD LARCH	(LARIX DECIDUA)	79
RB---RECENT BURN	(P. RADIATA)	1
OP---OLD PINE	(P. RADIATA)	39
YP---YOUNG PINE	(P. RADIATA)	1
YB---YOUNG BLACK WATTLEWOOD	(ACACIA MELANOXYLON)	3
YD---YOUNG DOUGLAS-FIR[2]	(PSEUDOTSUGA MENZIESII)	9

[1]SAME SITE AS EP BUT SOIL SAMPLES TAKEN FROM DIRECTLY UNDER EUCALYPTS.
[2]YB AND YD ADJACENT AREAS. PREVIOUS STAND; CORSICAN PINE (HARVESTED:1972).

TABLE 2. MYCORRHIZAL STATUS OF E. REGNANS GROWN IN FOREST SOILS FOR 4 ½ MONTHS.

SITE	# ECTO TIPS[1] TYPE 1	# ECTO TIPS[1] TYPE 2	ENDO[2] (%)	# NONMYC TIPS
EP	2	1	38	2
UE	1	2	17	2
OL	1	0	27	4
RB	0	0	8	6
OP	0	0	36	4
YP	0	0	12	4
YB	0	3	36	2
YD	4	0	0	2

[1]VALUES ARE AVERAGE VALUES PER ONE CM ROOT SEGMENT (25 SEGMENTS/PLANT).
[2]VALUES AS ABOVE BUT EXPRESSED AS % SEGMENT FILLED WITH FUNGAL MATERIAL.

TABLE 3. DRY WEIGHTS AND HEIGHTS OF CONTROL, ENDOMYCORRHIZAL AND ECTOMYCORRHIZAL E. REGNANS SEEDLINGS AT 4 MONTHS.

INOCULUM	LEAF DRY WT. (MG)	ROOT DRY WT. (MG)	TOTAL DRY WT. (MG)	HEIGHT (CM)
CONTROL	673A[1] (±109)[2]	283A (±58)	1138A (±190)	15.4A (±2.2)
GIGASPORA MARGARITA (ENDO)	906A (± 78)	403A (±60)	1517A (±153)	17.6AB (±1.7)
LACCARIA LACCATA (ECTO)	1239B (± 41)	590B (±65)	2120B (±115)	21.6B (±0.8)

[1]VALUES REPRESENT AVERAGE VALUES (N=10). VALUES IN COLUMNS FOLLOWED WITH SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT AT THE 0.05 LEVEL AS DETERMINED BY THE FISHER'S PROTECTED L.S.D..
[2](±STANDARD ERROR).

MYCORRHIZAE OF ANNUAL HELIANTHEMUM SPECIES FORMED WITH DESERT TRUFFLES

By

A. Alsheikh

Keywords--Helianthemum, Terfezia, Tirmania, helianthemoid mycorrhiza, desert truffles

Introduction

The annual Helianthemum species are endemic plants in the Arabian Peninsula and North Africa. H. ledifolium (L.) Mill. and H. salicifolium (L.) Mill. are most abundant in sites where desert truffles grow. Terfezia boudieri Chatin, T. claveryi Chatin, Tirmania nivea (Desf.) Trappe, and T. pinoyi (Maire) Malencon are four truffle species that form mycorrhizae with the two annual helianthemums and some other desert annual plants. Mycorrhizae formed by annual Helianthemum spp. inoculated with four desert truffle species have been described (Awameh and Alsheikh, unpublished data, Chevalier et al. 1984, Agronomie, 4(2):210). The mycorrhizal affinity of the other desert annuals has yet to be determined.

It is not clear yet if the type of "helianthemum mycorrhiza" formed with Terfezia spp. (Terfeziaceae) differ from those formed with Tirmania spp. (Pezizaceae), or to what degree the two genera are physiologically similar.

Methods and Materials

Desert truffle mycorrhizal roots were collected from Alshagayah and Alsalmi of southwest Kuwait from the Dibdibah formation during a field survey in February 1978. Truffle samples were located by observing the cracks and curvature of the soil over the ascocarp. A hole, 40 cm deep and 40 cm in diameter, was dug around each ascocarp. The latter was then removed together with the surrounding substrata. The ascocarp, the helianthemum plants, soil and fine gravel held by the mycelium were all placed in water for three hours. The ascocarp and the plants and roots were freed by a light water jet. The ascocarps and the plants were identified by use of Ceruti 1960, Alsheikh and Trappe 1983, (TBMS, 81:83) Daoud and Sheikh 1974, (J. Univ. Kuwait (Sci.) 1:97) and Trappe, personal communication. Roots were cleaned by ultrasonic water bath and washed and preserved in FAA. Microtomed double-stained transverse and longitudinal sections were prepared for the successive development stages of the mycorrhizae. A Stereomicroscope was used for whole root examination, while microtomed slides were examined under compound microscope.

Results and Discussion

The annual helianthemum plants are 5-20 cm tall, have a root system 5-15 cm long, with tap root and secondary roots that normally terminate in a series of very fine-branched feeder rootlets. The branching depends on the age of the plant and the development stage. The final branches, 2-20 mm long, lack root hairs. The root system is dimorphic: (1) fine, white-transluscent, uncolonized roots, and (2) thick, light yellow-brown opaque roots heavily endomycorrhizal with very large, septate hyphae, 6-10 µm broad at septa, that fill most of the epidermal and cortical cells. Root color is transparent to white in the early development stage, then turns to a very light brown, often being affected by the color of adhering soil. The main root diam is ± 0.5 mm, while the finest rootlet is only 60 µm in diam. The rootlet consists of a narrow central stele surrounded by endodermis, 2-4 cortical layers and epidermis. A dense weft of septate hyphae grows over the root surface. This mycelial growth forms a layer of one or two hyphae on the rootlet surface without forming a sheath. In some cases penetration of the rootlet epidermal, then cortical cells takes place when the hyphae or "haustoria" extend inward from the external mycelium growing parallel to the rootlet epidermal cells. The hyphae inside the cortical cells branch from one cell to another. Often the hyphae penetrate out to the rhizoplane and re-enter other epidermal and cortical cells. When colonization is heavy, the epidermis is densely colonized with hyphae, the external mycelium forms a network that holds soil and gravel particles to the rootlets. The hyphal branches or "haustoria" from the rhizoplane penetrate the epidermis into the luman of cortical cells, forming many short curved and densely interwoven extensions, or hyphae masses, similar to the pelotons or coils of ericoid mycorrhiza. The hyphal masses frequently surround the host cell nucleus. The cortical cells are ± inflated, and the cell growth is restricted to a radial direction. Hypertrophy in this case is generally related to the mycorrhization. The main hyphal stem of the pelotons, or sometimes more than one, inside the cortical cell is ± 5 µm broad at septa, forming hyphal masses 18-37 x 26-58 µm, occupying 50-67 percent of the cell size. The endodermis and the stele are free from colonization, and the inner cortical layer is rarely colonized. The hyphae are always intracellular. The epidermal cells often collapse due to the heavy fungal colonization. However, in desert annuals the cortical layer collapses due to the development of secondary phloem layers. The fine rootlets are devoid of any mantle and Hartig net, as is typical of the ectendo—or ectomycorrhiza, or any vesicles or arbuscules representative of the VA mycorrhiza.

Although these mycorrhizae appear to resemble ericoid mycorrhizae, they form on compeltely different hosts (annuals in the order Guttiferae) and completely different habitats (deserts) than Ericaceae. Pending results of additional research, I prefer to term them "helianthemoid" rather than "ericoid" mycorrhizae.

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STRUCTURE OF HYSTERANGIUM - EUCALYPTUS ECTOMYCORRHIZAS

By

B. Dell and N. Malajczuk

Keywords--*Eucalyptus marginata*, *E. diversicolor*, *Laccaria laccata*, root dimensions, abundance, superficial mycorrhiza.

Introduction

An examination of *Eucalyptus diversicolor* (karri) and *E. marginata* (jarrah) root systems in forests of Western Australia have revealed a large proportion of the fine feeder roots to be enveloped by superficial mycelium, an association not typical of eucalypt ectomycorrhizas (as defined by Chilvers and Pryor 1965). A study was made of these roots associated with *Hysterangium* sporocarps and compared with pyramidal mycorrhizas of *Laccaria laccata*.

Methods and Materials

Roots in the field were exposed by gentle litter removal and surface areas of *Hysterangium* mats recorded. Sections of roots were obtained after Malajczuk *et al.* (1984) for structural observations and root parameter measurements.

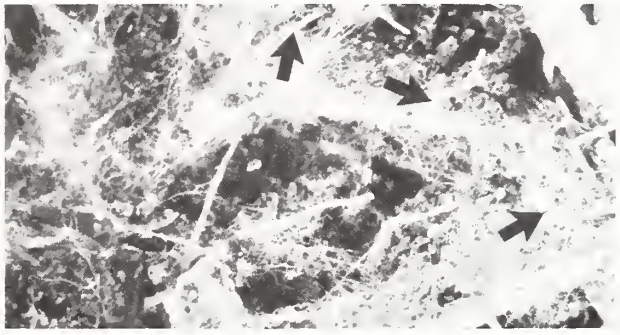

Figure 1. White *Hysterangium* mycelium around *Eucalyptus marginata* ectomycorrhizas (arrows); X 3.

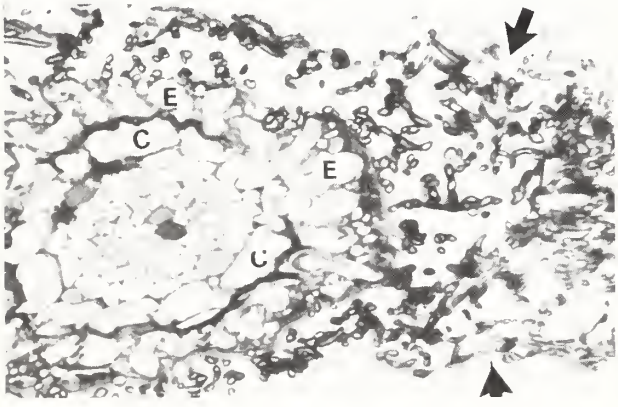

Figure 2. T.S. *Eucalyptus marginata* ectomycorrhiza. Note the mycelium (arrows) and unexpanded cortical (C) and epidermal cells (E); X 300

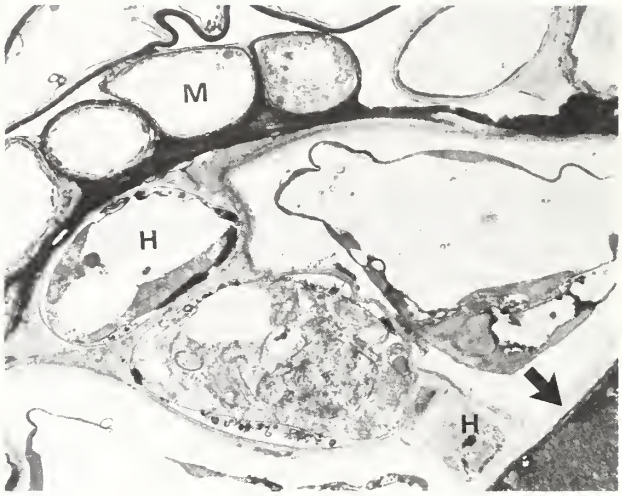

Figure 3. T.S. *Eucalyptus marginata* ectomycorrhiza showing mantle (M) and Hartig net (H) extending to the lignified hypodermis (arrow); X 8,200

Results and Discussion

In the karri forest *Hysterangium* mats occupied some 10% of the soil surface. Roots within the mats were enveloped with white mycelium but lacked pyramidal development (Fig. 1). Sectioned jarrah and karri roots from within the mats showed a diffuse mantle and a Hartig net penetrating to the hypodermis (Figs. 2,3). Unlike typical pyramidal sheathing mycorrhizas, the epidermal and cortical cells of *Hysterangium* mycorrhizas have dimensions similar to those of uninfected roots (Table 1). Apart from this feature, these roots have a mantle and Hartig net characteristic of ectomycorrhiza (Harley and Smith 1983). Because of their abundance in eucalypt forests further work on their significance in nutrient transfer is underway.

Table 1. Comparison of root parameters for mycorrhizal and non-mycorrhizal short *Eucalyptus* roots

Root type	Root diameter (μm)	Mantle thickness (μm)/no fungal cells	Radial diameter epidermal cells (μm)
(a)	159	-	21.5
(b)	260	26.6/7	40.6
(c)	155	3.4/2	20.4

(a) Uninfected root; (b) pyramidal sheathing mycorrhiza; (c) superficial *Hysterangium* mycorrhiza.

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MYCORRHIZAS OF WEST AFRICAN FOREST TREES

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Keywords - Caesalpinioideae, phosphorus

Introduction

A number of hypotheses have been advanced to explain the sporadic occurrence of ectomycorrhizas on tropical forest trees. It has been suggested that they are typically associated with one, or a combination of the following:

1. Certain angiosperm taxa, e.g. Dipterocarpaceae, Leguminoseae sub family Caesalpinioideae.
2. Low diversity stands.
3. Soils of low fertility.
4. Seasonal availability of nutrients and/or water.

These ideas are examined by reference to the mycorrhizas of some Ghanaian forest trees and to a recent floristic survey of rain forest in Cameroun (Gartlan et al., 1984). Two forest types were sampled in Ghana: mature moist semi-deciduous mixed forest at Kade, and dry forest of Talbotiella gentii at the southern end of the Volta lake. The Cameroun data come from rain forest in Korup forest reserve.

Results and Discussion

Kade

Kade is a floristically rich forest on soils of moderate fertility (available P 5-12 ppm). In a one-hectare sample plot there were 114 tree species and 580 stems > 30 cm girth at breast height. The position of all these stems was recorded. Five species of Leguminoseae sub family Caesalpinioideae were present. The position of the 16 individuals was noted and root samples were collected for assessment of mycorrhizal status (Table 1).

Table 1. Mycorrhizal status of some members of Leguminoseae sf Caesalpinioideae at Kade.

Tribe	Species	Ecto	VA
Caesalpinieae	Bussea occidentalis		X
Cassieae	Distemonanthus benthamianus		X
Detarieae	Afzelia bella	X	X
"	Copaifera salikounda		X
Amherstieae	Anthonotha macrophylla	X	

Afzelia bella, A. africana and Anthonotha macrophylla have previously been recorded as ectomycorrhizal in W. Africa (Redhead, 1980). Of the remaining 109 spp on the plot, 40 were either found to be VA or non-mycorrhizal or can safely be assumed to be so on the basis of previous collections in W. Africa. The status of 60 spp is unknown.

Individuals of A. macrophylla were somewhat clumped (R = 0.84) but the sample was small and many non-ectomycorrhizal large-seeded species behave in a similar fashion. Non-ectomycorrhizal Caesalpinioideae occur close to ectomycorrhizal species, often within their zone of root extension. Fruit bodies of a Russula sp and an unknown member of Hygrophoraceae were recorded in association with A. bella.

Dry forest

The Ghanaian endemic Talbotiella gentii (Caesalpinioideae; Detarieae) occurs as monospecific patches of 1 or 2 hectares in a matrix of mixed dry forest (Swaine & Hall, 1981). It is particularly associated with rocky outcrops on soils of varying nutrient status (Available P 13-27 ppm). Talbotiella forms VA-type mycorrhizas; its main competitors Drypetes parvifolia and Diospyros abyssinica were non-mycorrhizal. A closely related species in nearby forest, Hymenostegia afzelii (Caesalpinioideae; Detarieae) also forms VA-type mycorrhizas.

Cameroun

In their survey Gartlan et al (1984) enumerated over 40,000 trees of 400 spp in 135 sample plots and collected soil and other environmental data. When topographic effects were removed from the analysis of the data, floristic gradients were strongly correlated with concentration of available soil phosphorus. Species of sub family Caesalpinioideae, tribes Amherstieae and Detarieae, were strongly associated with soils of less than 5 ppm available phosphorus. Tetraberlinia bifoliata, T. moreliania, Didelotia africana and Microberlinia bisulcata were prominent. There is no direct evidence that these species are ectomycorrhizal but reference to related spp (Fassi & Fontana, 1962; P. Hogberg, personal communication) indicates that this is highly probable.

Conclusions

1. Not all members of Caesalpinioideae, even tribe Detarieae, form ectomycorrhizas, even when they occur in monospecific stands.
2. There is some evidence to link Caesalpinioideae, low soil phosphorus, and ectomycorrhizas however those species which form ectomycorrhizas do so even on soils of higher P status and even when they occur as isolated individuals in a matrix of VA forming species.
3. Those species which do not form ectomycorrhizas remain non-ectomycorrhizal even in close proximity to ectomycorrhizal species.

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Dr Valentin Furlan
Station de Recherches
2560, boul. Hochelaga
Sainte-Foy (Québec)
Canada G1V 2J3

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REGISTRATION FORM FOR THE THIRD EDITION ON THE INTERNATIONAL DIRECTORY OF MYCORRHIZASTS

EXCLUSIVELY for those not registered in the 2nd edition (1984) of the Directory

FORMULE D'INSCRIPTION POUR LA TROISIÈME ÉDITION DE L'ANNUAIRE INTERNATIONAL DES MYCORHIZOLOGUES

EXCLUSIVEMENT destinée aux non inscrits dans la 2e édition (1984) de l'annuaire

Family name/Nom de famille | Given name(s)/Prénom(s)

Address/Adresse

City/Ville | State/État or/ou Province | Postal Code/Code Postal

Country/Pays | TELEPHONE Code area/Code régional - Phone No./Nº de téléphone

Last degree received/ Dernier diplôme obtenu: 1 ☐ Ph.D. 2 ☐ D.Sc. 3 ☐ M.Sc. 4 ☐ B.Sc. 5 ☐ Other Autre _____

Position/Occupation:
1 ☐ Professor Professeur 2 ☐ Researcher Chercheur 3 ☐ Student Étudiant 4 ☐ Other Autre _____

FIELDS OF RESEARCH ON MYCORRHIZAE
DOMAINES DE RECHERCHE SUR LES MYCORHIZES

PLEASE: Indicate your principal fields of research on line no. 1, and minor fields on line no. 2. Choose code numbers of your fields of interest from list on reverse (maximum of 12 code numbers per line).

S.V.P.: Veuillez indiquer vos principaux domaines de recherche sur la ligne nº 1 et vos domaines de recherche secondaires sur la ligne nº 2 à partir des numéros code listés au verso (maximum de 12 numéros code par ligne).

1. ____, ____, ____, ____, ____, ____, ____, ____, ____, ____, ____, ____.

2. ____, ____, ____, ____, ____, ____, ____, ____, ____, ____, ____, ____.

Return a photocopy of this registration form duly filled before 31st December 1985 to:

Renvoyer une photocopie de cette formule d'inscription dûment remplie avant le 31 décembre 1985 à:

Dr Valentin Furlan
Station de Recherches
Agriculture Canada
2560, boul. Hochelaga
Sainte-Foy (Québec) Canada
G1V 2J3

LIST OF CODE NUMBERS OF THE FIELDS OF RESEARCH ON MYCORRHIZAE

LISTE DES NUMÉROS CODE DES DOMAINES DE RECHERCHE SUR LES MYCORHIZES

	ECTO	ENDO	ECTENDO	ORCHIDS ORCHIDÉES	ERICACEAE ÉRICACÉES
EVOLUTION	101	201	301	401	501
SYSTEMATICS SYSTÉMATIQUE	102	202	302	402	502
ECOLOGY ÉCOLOGIE	103	203	303	403	503
ANATOMY, MORPHOLOGY ULTRASTRUCTURE ANATOMIE, MORPHOLOGIE	104	204	304	404	504
PHYSIOLOGY PHYSIOLOGIE	105	205	305	405	505
BIOCHEMISTRY BIOCHIMIE	106	206	306	406	506
CULTURE (SPORES, MYCELIUM, TISSUE/TISSUS)	107	207	307	407	507
IMMUNOLOGY IMMUNOLOGIE	108	208	308	408	508
BIOLOGICAL INTERACTIONS INTERACTIONS BIOLOGIQUES	109	209	309	409	509
BIOCIDES	110	210	310	410	510
SOIL-PLANT RELATION RELATION SOL-PLANTE	111	211	311	411	511
NITROGEN FIXING SYMBIOSES SYMBIOSES FIXATRICES D'AZOTE	112	212	312	412	512
TROPICAL PLANTS PLANTES TROPICALES	113	213	313	413	513
APPLIED RESEARCH RECHERCHE APPLIQUÉE	114	214	314	414	514

NOTE: Some fields of research may not apply.

NOTE: Certains domaines de recherche peuvent ne pas s'appliquer.

USDA-ARS
Horticultural Crops Research Laboratory
3420 N.W. Orchard Avenue
Corvallis, OR 97330